



Planetary protection
of Outer Solar System

THE INTERNATIONAL PLANETARY PROTECTION HANDBOOK



PLANETARY PROTECTION
OF OUTER SOLAR SYSTEM

Planetary protection
of Outer Solar System

3

THE INTERNATIONAL PLANETARY PROTECTION HANDBOOK

The PPOSS project Coordination and Support Action has received funding from the European Commission's H2020 Program (2016-2018) under grant agreement 687373.

Table of Content

TABLE OF CONTENT	5
INTERNATIONAL LIST OF ACRONYMS	9
PLANETARY PROTECTION GLOSSARY	11
PPOSS CONSORTIUM – HANDBOOK AUTHORS	13
INTRODUCTION	15
HISTORICAL PERSPECTIVE	15
THE PLANETARY PROTECTION OF OUTER SOLAR SYSTEM (PPOSS) PROJECT	16
INTRODUCTION TO PLANETARY PROTECTION IN THE OUTER SOLAR SYSTEM, FROM MARS TO THE ICY MOONS	17
EXECUTIVE SUMMARY	17
WHY HAS PLANETARY PROTECTION SO FAR CONCENTRATED ON MARS?	17
WHY IS PLANETARY PROTECTION NOW LOOKING TO THE ICY MOONS?	18
HOW ARE MARS AND THE ICY MOONS PHYSICALLY SIMILAR?	18
HOW ARE MARS AND THE ICY MOONS PHYSICALLY DIFFERENT?	18
WHAT IS UNIQUE ABOUT THE MARS ENVIRONMENT?	19
WHAT IS UNIQUE ABOUT AN ICY MOON ENVIRONMENT?	19
HOW DIFFERENT ARE THE HABITABILITIES OF MARS AND THE ICY MOONS?	19
HOW DIFFERENT ARE THE POSSIBILITIES OF FALSE POSITIVES ON MARS AND THE ICY MOONS?	20
WHAT ARE THE STANDARD PLANETARY PROTECTION STRATEGIES CURRENTLY USED FOR MARS?	20
WHAT ARE THE STANDARD ORGANIC CLEANLINESS CONSIDERATIONS CURRENTLY USED FOR MARS?	21
WHAT ARE THE STANDARD PLANETARY PROTECTION STRATEGIES CURRENTLY USED FOR ICY MOONS?	21
WHAT EXISTING PLANETARY PROTECTION TECHNIQUES ARE PARTICULARLY USEFUL FOR ICY MOONS?	22
WHAT NEW PLANETARY PROTECTION TECHNIQUES NEED TO BE DEVELOPED FOR ICY MOONS?	23
WHAT ORGANIC CLEANLINESS CONSIDERATIONS ARE PARTICULARLY USEFUL FOR ICY MOONS?	23
WHAT MISSIONS ARE BEING PLANNED FOR THE ICY MOONS?	23
CONCLUSIONS	24
HANDBOOK OBJECTIVES	28
INTERNATIONAL PARTNERS	28
CHAPTER 1. PLANETARY PROTECTION BASICS	29
PLANETARY PROTECTION BASICS	29
<i>What planetary protection is not</i>	29
<i>History of planetary protection</i>	29
<i>Rationale for planetary protection</i>	29
<i>Framework for planetary protection</i>	30
<i>Planetary protection categories</i>	30
<i>Planetary protection requirements</i>	31
<i>Reporting to COSPAR</i>	35
<i>Things to remember</i>	35
THE COSPAR PANEL ON PLANETARY PROTECTION	37
<i>COSPAR at a Glance</i>	37
<i>The Framework For Planetary Protection</i>	37
<i>The COSPAR Panel on Planetary Protection</i>	37
<i>Maintaining the COSPAR Planetary Protection Policy</i>	38
<i>Things to remember</i>	39
BASICS OF MICROBIOLOGY	40
<i>Microorganisms are the oldest form of life on Earth</i>	40
<i>Size, shape and structure of microorganisms</i>	41
<i>Microbial distribution and abundance</i>	43

<i>The Detection of Microorganisms</i>	44
<i>Things to remember</i>	45
ORGANIC CONTAMINATION CONTROL	47
<i>Organic contamination</i>	47
<i>Importance of organic contamination control</i>	47
<i>Practices of organic contamination control</i>	48
<i>History of organic contamination control</i>	49
<i>Current challenges in organic contamination control</i>	49
BIOBURDEN, ASSAY AND STERILIZATION	51
<i>Microbial Diversity</i>	51
<i>Bioburden on Space craft</i>	52
<i>Sterilization Technologies and Bioburden Reduction</i>	53
<i>Accounting and Assay</i>	55
<i>Things to remember</i>	55
ORBITAL DYNAMICS AND IMPACT PROBABILITY ANALYSIS	57
<i>Overview</i>	57
<i>Hayabusa-2 and Planetary Protection</i>	57
<i>How to Prove Compliance?</i>	58
<i>Failure Probability: Spacecraft system failure rate</i>	58
<i>Failure probability: Meteoroid kill rate</i>	59
<i>Impact probability after failure</i>	59
<i>Summary</i>	64
CHAPTER 2. CASE STUDIES	65
PLANETARY PROTECTION CATEGORY II	65
<i>Planetary Protection Category II Description</i>	65
<i>Case Study for Planetary Protection Category II: BepiColombo</i>	65
<i>Requirements for Case Study</i>	65
<i>Things to Remember</i>	65
PLANETARY PROTECTION CATEGORY III	67
<i>Planetary Protection Category III Description</i>	67
<i>Case Study for Planetary Protection Category III: ExoMars Trace Gas Orbiter (TGO)</i>	67
<i>Requirements for Case Study</i>	67
<i>Implementation of Requirements for Case Study</i>	67
<i>Things to Remember</i>	68
PLANETARY PROTECTION CATEGORY IVA – INSIGHT	70
<i>Category IVa Case Study Overview</i>	70
<i>Planetary Protection Category IVa Description</i>	70
<i>Case Study for Planetary Protection Category IVa – Insight</i>	70
<i>Requirements for the INSIGHT Mission</i>	71
<i>Implementation of Requirements</i>	72
<i>Things to Remember</i>	74
<i>Acknowledgements</i>	74
PLANETARY PROTECTION CATEGORY IVb – EXOMARS	75
<i>Planetary Protection Category IVb Description</i>	76
<i>Planetary Protection Category IVb – The ExoMars mission</i>	76
<i>Planetary Protection Requirements for ExoMars</i>	76
<i>Implementation of Planetary Protection Requirements for ExoMars</i>	77
<i>Planetary Protection Requirements verification</i>	85
<i>Launch Campaign recontamination on ground and at launch</i>	85
<i>Planetary Protection documentation</i>	86
<i>Things to Remember</i>	86
PAYLOAD CASE STUDY	87
CASE STUDY FOR PLANETARY PROTECTION CATEGORY V – UNRESTRICTED EARTH RETURN: HAYABUSA-1 & 2	91
<i>Planetary Protection Category V Description</i>	91
<i>Requirements for Case Study</i>	92
<i>Implementation of Requirements for Case Study</i>	96

<i>Things to Remember</i>	96
CHAPTER 3. LESSONS LEARNED – HOW TO COMPLY WITH PLANETARY PROTECTION STANDARDS	99
COMMON PROBLEMS IN THE CHAIN OF COMMAND (MANAGEMENT/COORDINATION)	99
<i>Problem 1: An unclear chain of command with part of the chain left without a link to the Planetary Protection Officer</i>	99
<i>Problem 2: Planetary Protection relevant personnel not included at key organizational project meetings</i>	99
<i>Problem 3: Lack of PP supervision along the entire project</i>	99
<i>Problem 4: Lack of links between the different levels managing PP requirements</i>	99
<i>Problem 5: Out of the loop sub-contractors</i>	100
<i>Problem 6: Lack of coordination between Project PP Team and QA/QC staff</i>	100
PROBLEMS ON WHEN/HOW TO IMPLEMENT PLANETARY PROTECTION REQUIREMENTS	100
<i>Problem 7: Great variability in where the PP function lays /resides within a project</i>	100
<i>Problem 8: Planetary Protection not taken into account during Mission Design</i>	100
COMMON PROBLEMS DUE TO LACK OF TRAINING	100
<i>Problem 9: A project manager, mission manager or even PP Team lead with no previous solid PP training</i>	100
<i>Problem 10: Not enough specific training</i>	100
<i>Problem 11: Contractors not aware of what PP is and how it may influence their task</i>	101
BUDGET OVERRUNS	101
<i>Problem 12: Project Financiers not aware of PP requirements</i>	101
<i>Problem 13: Difficulty in estimating an exact PP budget, with challenges in accounting peripheral expense.</i>	101
<i>Problem 14: Costs for operating the cleanroom and garments, additional and special tools and lab instruments, consumables, personnel etc. were massively underestimated.</i>	101
DELAYS	101
<i>Problem 14: Time for any contingencies is not taken into account when planning the project PP calendar.</i>	101
<i>Problem 15: Working time in cleanrooms strongly underestimated (due to the different phases: cleaning/assembly/ testing but also due to staff behavior. Staff need to adapt to the regulatory procedures within cleanrooms to make sure no re-contamination occurs.</i>	101
<i>Problem 16: Underestimated the packing of hardware difficulty for transport and tests</i>	101
<i>Problem 17: When designs change, PP requirements may/will change.</i>	102
COMMON ERRORS	102
<i>Problem 18: Overlap/misunderstanding/interference between PP and CC requirements.</i>	102
<i>Problem 19: Short-staffed PP teams. (right amount not requested or not approved by project manager)</i>	102
<i>Problem 20: Wrong choice of test facilities.</i>	102
<i>Problem 21: Wrong selection of material.</i>	102
<i>Problem 22: Training being planned too early or too late along the project.</i>	102
<i>Problem 23: Stand-alone Planetary protection Documentation.</i>	102
CHAPTER 4. PLANETARY PROTECTION CHECK LIST	103
PLANETARY PROTECTION CHECK LIST	103
<i>Plan a first meeting with your PPO during which you will:</i>	103
<i>Next, plan an early PP meeting with all agents involved within the mission.</i>	103
<i>Designate one person per project to carry the responsibility of acting as the designated link regarding PP issues at all stages of the project.</i>	103
<i>Discuss technical aspects such as:</i>	103
<i>Discuss the PP budget</i>	104
<i>Plan the PP project schedule, featuring:</i>	104
<i>Take into account that there will be, more often than not, contingencies</i>	104
<i>Discuss possible changes, such as if hardware redesign is being considered. This way, alternative PP procedures can be prepared in advance and will avoid over costs or delays.</i>	104
<i>For Category IV and V missions</i>	104
WORKING WITH YOUR PLANETARY PROTECTION OFFICER	104
<i>Contact your PPO to:</i>	104
<i>Contact your PPO for:</i>	105
GENERAL RECOMMENDATIONS.....	105
<i>To avoid delays:</i>	105



ANNEXES	107
PLANETARY PROTECTION AT ESA	107
ESA PLANETARY PROTECTION POLICY	107
ESA PLANETARY PROTECTION REQUIREMENTS	107
ESA PLANETARY PROTECTION ORGANIZATION.....	107
ESA PLANETARY PROTECTION RESPONSIBILITY	108
THINGS TO REMEMBER.....	108
PLANETARY PROTECTION AT NASA.....	109
HISTORICAL DEVELOPMENT.....	109
ORGANIZATION OF PLANETARY PROTECTION WITHIN NASA.....	109
DOCUMENT HIERARCHY	110
IMPLEMENTATION HISTORY	110
PLANETARY PROTECTION MANAGEMENT IN JAXA	112

International List of Acronyms

AIT	Assembly, Integration and Test
ATLO	Assembly, Test and Launch Operations
BPOCC	Best Practice of Organic Contamination Control
CAD	Computer Aided Design
CAST	China Academy of Space Technology
CETEX	Committee on Contamination by Extraterrestrial Exploration
CISAS	Centro di Ateneo di Studi e Attività Spaziali “Giuseppe Colombo”
CNES	Centre National d'Études Spatiales (English: French National Centre for Space Studies)
COPUOS	Committee on the Peaceful Use of Outer Space
COSPAR	Committee on Space Research
CTE	Coefficient of Thermal Expansion
DHMR	Dry Heat Microbial Reduction
DLR	German Aerospace Center
DREAMS	Dust characterization, Risk assessment and Environment Analyzer on the Mars Surface
ECSS	European Cooperation for Space Standardization
ESA	European Space Agency
GMP	Good Manufacturing Practices
IAF	International Astronautical Federation
ICSU	International Council of Scientific Union
ISO	International Organization for Standardization
JAXA	Japan Aerospace Exploration Agency
JPL	Jet Propulsion Laboratory
MER	Mars Exploration Rover
MGS	Mars Global Surveyor
MOC	Molecular Contamination (on hardware)
MOMA	Mars Organic Molecule Analyzer
MPF	Mars Pathfinder
MRO	Mars Reconnaissance Orbiter
MSR	Mars Sample Return
NAS	National Academies of Sciences (USA)
NASA	National Aeronautics and Space Administration
NRC	National Research Council
PAC	Particulate Contamination (on hardware)

PCR	Polymerase Chain Reaction
PP	Planetary Protection
PPO	Planetary Protection Officer
PPR	Planetary Protection Requirements
PRR	Preliminary Requirement Review
QA/QC	Quality Assurance/ Quality Control
Roscosmos	Also known as the Roscosmos State Corporation for Space Activities, is the Russian governmental body responsible for the space science program and general aerospace research
SAM	Sample Analysis at Mars
SB	Small Body
SME	Small and Medium Enterprises
SRR	System Requirements Review
SSB	Space Studies Board
STP	Standard Temperature and Pressure
TAS	Thales Alenia Space
TEC-Q	Head of ESA Product Assurance and Safety Department

Planetary Protection Glossary

Decontamination	Removal of contaminating agents, e.g., microorganisms or organic compounds
Disinfection	Elimination of nearly all recognized pathogenic microorganisms but not necessarily all microbial forms
Encapsulated bioburden	Bioburden encapsulated in non-metallic material, protected from gas exchange
Exposed surfaces	Internal and external surfaces free for gas exchange
Extant life	Form of life, or signatures thereof, that is viable today whether metabolically active or dormant
Extinct life	Form of life, or signatures thereof, that is unambiguously no longer metabolically active or dormant
Inbound leg	(In the frame of sample return missions) Part of the mission returning to Earth
Mated surfaces	Surfaces joined by fasteners rather than by adhesives
Outbound leg	(In the frame of sample return missions) Part of the mission leaving Earth
Planetary Protection Officer	Within a space agency, the PPO oversees compliance with policy, including providing requirements and verification, working closely with the Project Manager to implement the established requirements to achieve compliance with policy
Planetary Protection Mission/Program Manager	Responsible for project compliance, scheduling, negotiating/overseeing implementation and documentation of all planetary protection activities. Ensures that the project met the PP requirements and was granted permission to launch
Restricted Earth Return	Planetary protection sub-Category V for sample return missions from Solar system bodies deemed by scientific opinion to have a chance of harboring indigenous life forms
Sterilization	A validated process used to render, through elimination (through removal, inactivation or killing) of all living microorganisms and viruses, a product “free” from viable microorganisms
Unrestricted Earth Return	Planetary Protection sub-Category V for sample return missions from Solar system bodies deemed by scientific opinion to have no indigenous life forms

PPOSS Consortium – Handbook Authors

Editorial Team



Alissa Haddaji
Handbook Coordinator



Gerhard Kminek
Supervisor



Jean-Louis Fellous
Handbook Editor

Contributors from the PPOSS Consortium



Mark Sephton



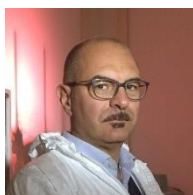
Sam Royle



Petra Rettberg



Elke Rabbow



John Brucato

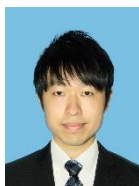


Susan McKenna-Lawlor

External Contributors



Hajime Yano



Toshihiro Chujo



J Andy Spry



Diana Margheritis

Introduction

Historical Perspective

As J. Lederberg and D.B. Cowie explain:

“...we are in the awkward situation of being able to spoil certain possibilities for scientific investigations for a considerable interval before we can constructively realize them...we urgently need to give some thought to the conservative measures needed to protect future scientific objectives on the moon and the planets...” J. Lederberg and D.B. Cowie, *Science*, 1958.

Planetary Protection serves to preserve planetary conditions for future scientific investigations and is the most effective way to protect space science and exploration. Its regulation concerning sample return missions aims at protecting the Earth and its biosphere (including the Moon) from potential harmful extraterrestrial biologic and organic contamination.

It responds to concerns raised by the scientific community (namely the International Astronautical Federation (IAF), the COPUOS and the US National Academy of Science), that space missions might compromise future scientific exploration, if not handled carefully. In 1958, the International Council of Scientific Unions (ICSU), now known as the International Council for Science, established an ad-

hoc Committee on Contamination by Extraterrestrial Exploration (CETEX) and adopted its Code of Conduct. ICSU established COSPAR in 1958 and transferred the CETEX mandates to the newly founded Committee.

In 1961, the Ranger missions were the first spaceflight missions to use the Code-of-Conduct. The United Nations Outer Space Treaty’s (1966) article IX establishes the legal basis for Planetary Protection. It stipulates: *“... parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extra-terrestrial matter and, where necessary, shall adopt appropriate measures for this purpose...”*

COSPAR established the Consultative Group on Potentially Harmful Effects of Space Experiments, has provided an international forum to discuss such matters and then formulated a policy (with implementation requirements as an international standard which now serves as a guide of compliance with Article IX of the UN Space Treaty in that area. Since then, all planetary missions have had to implement planetary protection measures at different degrees – ranging from simple documentation to terminal sterilization of entire flight systems.

The Planetary Protection of Outer Solar System (PPOSS) Project

16

The Planetary Protection of Outer Solar System (PPOSS) project tackles the science, technology and policy making components related to biological and organic contamination of outer solar system bodies, in particular icy moons. This intensive three-year program provides an international platform and forum where science, industry and policy actors meet to nurture and catalyze discussions, exchange of knowledge and produce policy recommendations on the matter of planetary protection.

The main objectives of the PPOSS project are:

- Describe the state of the art and good practice for implementing planetary protection requirements, and identify good practices and lessons to be learnt.
- Identify scientific challenges, requirements and knowledge gaps related to planetary protection of outer solar system bodies, including small solar system bodies.
- Develop a European engineering roadmap for the industry sector.
- Review the international outer solar system planetary protection regulation structure and categorization and suggest improvements, and,
- Facilitate the dissemination of knowledge related to planetary protection. The PPOSS project, gathers seven European partner organizations, one international partner and one international observer.

Kicked off in January 2016, PPOSS is supported by the European Commission Horizon 2020 program under grant agreement 687373.

For more information: <http://pposs.org>

Introduction to Planetary Protection in the Outer Solar System, from Mars to the Icy Moons

EXECUTIVE SUMMARY

Planetary protection aims to limit the harmful contamination of habitable worlds beyond Earth, by terrestrial organisms and organic molecules. The recent shift in focus of astrobiological exploration from just Mars to include the icy moons of the outer solar system necessitates review and expansion of our planetary protection protocols. There are significant differences between Mars and the icy moons: the former is dry and organic matter-poor (at least at the surface) while the latter are potentially wet and organic matter-rich. Contamination at Mars can be expected to comprise local isolated events, although storms and dust transport do involve planet wide atmospheric transport. On the other hand, contamination of subsurface oceans on icy moons can potentially become global. The production of false positives on life detection missions to Mars and the icy moons are likely to be different in nature and frequency. Mars life detection experiments are more likely to suffer from terrestrial contamination whereas those on icy moons are subject to being overwhelmed by non-biological mimics of biopolymers.

WHY HAS PLANETARY PROTECTION SO FAR CONCENTRATED ON MARS?

So far the search for life beyond the Earth has focused on Mars. The Viking landers in the 1970's looked directly for life and the later Curiosity lander is looking for organic compounds which may provide evidence of past or present life (Grotzinger *et al.*, 2012; Klein, 1976; Leshin *et al.*, 2013; Levin and Straat, 1976; Levin and Straat, 1979; Ming *et al.*, 2014). The lure of Mars exploration reflects its proximity to Earth and the possibility of its past habitability.

Studies of biology in extreme environments on Earth continue to expand the known range of environmental parameters compatible with life, increasing our knowledge about the likelihood of organisms surviving in isolated circumstances on Mars.

Present day habitability is a possibility for a number of areas on the red planet identified as potential 'special regions' (Rummel *et al.*, 2014). These locations (should any exist) may accommodate the survival and replication of terrestrial microbes (Rettberg *et al.*, 2015; Rettberg *et al.*, 2016). An environment of biotic relevance may be as small as 10 to 1,000 microns. Thus, habitable conditions need only

be restricted to that area/s with which microbial life is in direct contact. Seven environments which may exist and be important to sensitivity of contamination by terrestrial life (and by similarity, may also be important for present-day extant life on Mars) have already been documented and include: extreme conditions with liquid brines; underground shelters and impact related hydrothermal systems (Rummel *et al.*, 2014).

The delivery of terrestrial microbes to Mars and their subsequent survival and proliferation can preclude the discovery of pristine evidence of bona fide Martian life. Fortunately, the most common terrestrial microorganisms that may contaminate spacecraft can be expected to be killed off by the radiation exposure, high temperatures and desiccation associated with spaceflight. Yet it must be noted that studies have shown that some microorganisms (especially spores) display adaptations to desiccated and high radiation environments (Musilova *et al.*, 2015) and may survive space travel (Schuerger *et al.*, 2003; Tauscher *et al.*, 2006; Vaishampayan *et al.*, 2010). It is noted that, even if rendered non-viable by exposure to spaceflight conditions, dead microbes are still composed of organic compounds that could obfuscate analysis of native data collected on Mars.

The rolling wave of current Mars exploration has several implications for causing the forward contamination of Mars. First, the number of planned Mars missions alone increases the potential for contamination. Second, the scope of activities conducted during surface missions and the number of known potentially habitable environments to be explored in the near future are expected to increase dramatically and will target regions deemed likely to provide evidence of past or present water (liquid or ice) (NRC 2006).

WHY IS PLANETARY PROTECTION NOW LOOKING TO THE ICY MOONS?

There is increasing evidence of the potential for life to exist on the icy moons of our outer Solar System. Subsurface oceans may be common on icy moons. Magnetic field data suggest the presence of highly conductive saline water below the surface of Europa (Kivelson *et al.*, 2000), Ganymede (Kivelson *et al.*, 2002) and Callisto (Kivelson *et al.*, 2000).

It was recently announced (2016) in *Nature News* that observations led by W. Sparks using the Hubble Space Telescope had three times observed plumes of, what is suspected to be, water vapor emanating from the surface of Europa. These observations, which are ongoing (Sparks *et al.*, 2016) support earlier reports by Roth *et al.* (2014) of the possible presence of water plumes at Europa which, if linked to a subsurface ocean, could potentially form conduits to a regime that supports life.

Recently the Cassini mission detected the expulsion of hydrothermally driven plumes from Enceladus (Porco *et al.*, 2006; Hsu *et al.*, 2015; McKay *et al.*, 2014; Spencer and Nimmo, 2013). The possibility of subsurface water on the icy moons has implications for the origin and distribution of life in the solar system because liquid water is a key prerequisite for habitability (Sephton 2004). However, the case that the icy moons might provide a source of life is by no means universally accepted (Pascal, 2016). Numerous lander types (including vehicles with the capability to penetrate through ice to potential subsurface oceans) and

flyby missions are being studied or developed to search for life on the icy moons. The associated potential to either contaminate indigenous biota or create false positives in life detection experiments is enhanced by the presence of water.

HOW ARE MARS AND THE ICY MOONS PHYSICALLY SIMILAR?

Mars and the icy moons of the outer solar system share some physical properties. Both are irradiated by the Sun and the icy moons receive in addition particle radiation from the magnetospheres of the planets they orbit. This radiation leads to the production of highly oxidized surfaces (Klein, 1978; Raulin *et al.*, 2010). Both Mars and the icy moons contain requisite abundances of all the elements necessary for life (Kargel *et al.*, 2000; Zolotov, 2007; Zolotov and Shock, 2001). Hydrothermal systems, often cited as locations of astrobiological interest, are present on Mars (Cockell and Barlow, 2002) while ocean-floor interactions are argued to occur on the icy moons (McCullom *et al.*, 1999; Raulin *et al.*, 2010; Vance *et al.*, 2007).

HOW ARE MARS AND THE ICY MOONS PHYSICALLY DIFFERENT?

Mars is relatively close to the Earth and can be reached by spacecraft in about six months. Icy moons take longer to reach by spacecraft (Voyager arrived at Jupiter in two years but Juno took five years to arrive at Jupiter and Cassini took seven years to arrive at Saturn). Mars is bathed in galactic and solar radiation (Kminek and Bada, 2006) but the radiation levels present at the surface on Mars are less than those experienced by some icy moons (Bagenal *et al.*, 2015). Mars has high levels of dust while the icy moons have large areas of water ice. Excluding surface processes, Mars can be considered geologically 'dead' with a stagnant lithosphere. In contrast, tidal interactions on Icy Moons may drive active cryospheres that facilitate chemical cycles with the capability to exchange surface material with a subsurface ocean (Hand *et al.*, 2007; Phillips *et al.*, 2000; Porco *et al.*, 2006).

WHAT IS UNIQUE ABOUT THE MARS ENVIRONMENT?

Mars displays seasonal changes owing to its obliquity of 25.19° which lead to variable polar ice caps. The topography and thermophysical properties of the Martian surface generate temperature and pressure gradients which drive local and regional scale atmospheric circulation, leading to the production of dust storms (Cantor *et al.*, 2001). There are rocks and minerals at the surface of Mars but, while there is plenty of evidence of a past active plate tectonic system, much like that on Earth, displaying frozen preserved subduction and spreading zones, transform faults and volcanoes, Mars is now tectonically inactive. Plate tectonics ceased when the mantle had cooled too extensively to support constructive plate margin spreading (Sleep, 1994).

WHAT IS UNIQUE ABOUT AN ICY MOON ENVIRONMENT?

Liquid water, the primary prerequisite for habitability, is likely to be present at the icy moons Europa (Kivelson *et al.*, 2000), Ganymede (Kivelson *et al.*, 2002), Callisto (Kivelson *et al.*, 2000) and Enceladus (Hsu *et al.*, 2015). Tidal energy drives the icy moon geological and geochemical exchange process which may promote interactions between a subsurface ocean/its rocky core and a chemically-rich oxidized moon surface (Raulin *et al.*, 2010). Cryovolcanic plumes which release water ice, gases and organic compounds (Hansen *et al.*, 2006; Porco *et al.*, 2006; Waite Jr *et al.*, 2009) are known to be present on at least two icy moons.

HOW DIFFERENT ARE THE HABITABILITIES OF MARS AND THE ICY MOONS?

Liquid water is an essential requirement for all known terrestrial life. Biochemistry relies on a medium in which compounds can dissolve and chemical reactions occur. Few other solvents can match the capability of water to sustain the reactions of life under conditions which support a liquid phase – which is neither too cold

to inhibit biochemical reactions from being active, nor too hot to break the bonds of organic compounds (Sephton, 2004). Habitability on Mars is likely to be confined to subsurface aquifers and hydrothermal regions or highly heterogeneous and transient surface water sources, perhaps related to deliquescent salts (Gough *et al.*, 2014), owing to the highly arid nature and UV flux of the solar-exposed surface environment. Despite showing abundant evidence of past habitable conditions, the current day Mars surface environment appears relatively challenging for life. The icy moons contain liquid water (Hsu *et al.*, 2015; Kivelson *et al.*, 2000; Kivelson *et al.*, 2002) and therefore present potentially habitable environments for any still viable microbes brought from Earth in a forward contamination event. Survival and proliferation of organisms is thus plausible, posing the need for careful planetary protection consideration.

Extant life on Mars or the icy moons is likely to be subsurface and therefore have to rely on energy sources independent of oxygen and surface photosynthesis. Putative extant biospheres would therefore likely be supported by chemotrophic primary producers. For example, aquifers of acidic brines could provide the ingredients needed to support metabolic pathways for subsurface Martian organisms, sustained by iron and sulphur chemolithotrophy; analogous to the subsurface anaerobic microbial habitat of the Rio Tinto deposits (Fernández-Remolar *et al.*, 2008). The oxidation of seismogenic H_2 is thought to be a major source of metabolic energy in the deep subsurface on Earth and could similarly support any extant biosphere on Mars (McMahon *et al.*, 2016).

In the high radiation environment of the icy moons, Oxidants produced by radiation on the surface of icy satellites could be carried to subsurface liquid water reservoirs that may contain reductants (redox reactions produce an energy source, see McKay *et al.*, 2008 and references therein). In a closed system consisting of a moon covered by thick ice, water-rock reactions in a tidally heated reservoir or a series of redox reactions produced by the radioactive

decay of long-lived radioisotopes could produce H₂, which, together with CO₂, would provide an energy source for methanogenic or sulphur reducing ecosystems (McKay *et al.*, 2008). Also, a radiation-driven ecosystem could exist with oxidants and organics suitable as energy sources produced in H₂O/CO₂ ices by radiation chemistry (Chyba, 2000).

HOW DIFFERENT ARE THE POSSIBILITIES OF FALSE POSITIVES ON MARS AND THE ICY MOONS?

False positives are generated when a signal is detected that can be confused with signals from biological materials. On Mars, organic compounds are likely to be scarce today and the major challenge lies in detecting them. Non-biological organic compounds can originate from meteoric or cometary material or through synthesis in hydrothermal systems. These organic carbon species can thereafter be transformed via chemical processes which are not, however, well understood (Mahaffy *et al.*, 2004). The radiation environment of Mars can promote the survival of small organic compounds (Pavlov *et al.*, 2012). On icy moons, organic compounds are likely to be plentiful and the major challenge lies in diagnosing their source. Polymerization of simple organic compounds is favored under conditions thought to exist on icy moons (Kimura and Kitadai, 2015). The effects of radiation on pre-existing natural organic mixtures have been studied (Court *et al.*, 2006). Radiation alteration of complex hydrocarbon mixtures in and oxygen-free environments leads to free radical generation and a decrease in the average size and extent of alkylation of polycyclic aromatic hydrocarbons; an increase in the abundance of oxygen-containing compounds; irradiation of methane produces polymerization and organic solids, with progressively increasing average size and degree of alkylation for their constituent aromatic units (Court *et al.*, 2006). De novo synthesis is also possible and high radiation environments may foster the production of amino acids and their oligomers, polymers or macromolecules (Cassidy *et al.*, 2010; Neish *et al.*, 2010; He and Smith, 2014). Thus, on Mars the low abundances of organic compounds could

lead to false positives from terrestrial contamination, while, on icy moons, the detection of polymers and macromolecules could lead to false positives when abiogenic polymers are confused with biopolymers.

WHAT ARE THE STANDARD PLANETARY PROTECTION STRATEGIES CURRENTLY USED FOR MARS?

Spacecraft that fly by or enter orbit around Mars are subject to planetary protection requirements (Category III) designed to control contamination and to reduce the risk that a spacecraft or its boosters will impact the planet. The spacecraft are assembled in clean rooms rated at Class 100,000 (ISO 8) or better (i.e., less than one hundred thousand particles in the size range up to 0.5 µm for every cubic foot of air; (ISO, 2016), and it is ensured that the probability of impact by the launch vehicle and the flyby spacecraft does not exceed 10⁻⁴ and 10⁻², respectively, over a time period of 50 years. The lifetime of an orbiter must be such that it remains in orbit for a period in excess of 20 years from launch, with a probability of impact of 0.01, and the probability of impact during the next 30 years must be no higher than 0.05. If the orbital lifetime requirements cannot be met, then the surface microbial bioburden must be fewer than 3 × 10¹⁵ total surface spores. Following bioassay to confirm that the low surface bioburden requirement is met, such spacecraft must be protected against recontamination.

Spacecraft that land on Mars but are not equipped with life-detection experiments are subject to planetary protection requirements designed to control the lander's bioburden and to prevent accidental impact by hardware not intended to land. The total probability of any accidental impacts by any hardware other than the lander must be no more than 10⁻⁴. Bioburden control involves assembly in a Class 100,000 (ISO 8) or better clean room, the implementation of periodic microbiological assays, and the maintenance of hardware cleanliness. Bioburden reduction to the level of 300,000 spores per landing event is required.

The mission team is also required to provide inventory, documentary, and archive samples of organic compounds used in the construction of the lander and associated hardware that might accidentally impact the planet. Finally, the locations of landing sites and impact points must be assessed as accurately as possible, and the condition of the hardware at each site must be estimated to assist in determining the potential location of organic compounds. Recently, the ESA lander Schiaparelli crashed on Mars with almost full thruster propellant tanks and led to the unexpected explosive deposition of large amounts of fuel on the planetary surface (ESA, 2016).

If such an event occurred in a ‘special region’ of Mars or into water on an icy moon, this level of irreversible contamination could be severely damaging to future scientific results.

WHAT ARE THE STANDARD ORGANIC CLEANLINESS CONSIDERATIONS CURRENTLY USED FOR MARS?

Evidence of past or present life can be revealed by the detection of organic compounds that result from biochemical processes. Achieving effective organic compound detection in environments where indigenous organic contents may be very low requires extremely low levels of organic terrestrial contamination/organic cleanliness (Blakkolb *et al.*, 2014). Organic cleanliness has to be maintained during each step in manufacturing, assembly, transport, sample processing and analysis. Most crucially organic cleanliness is required during sample collection, processing, and delivery to the analytical instrument.

The Mars Science Laboratory mission set a limit of 40 parts per billion (ppb, by weight) total terrestrial organic contamination load per sample analyzed by the Sample Analysis on Mars instrument. In detail, the Mars Science Laboratory activities subdivided the 40 ppb into flight system components to instrument sources in a 9:1 ratio. The most important sources of terrestrial contamination in samples were determined to be surfaces of the sample

collection and handling equipment, including the drill.

To achieve a high level of organic cleanliness aboard the Mars Science Laboratory, the individual hardware piece parts of the sample transfer chain were treated prior to assembly using acetone and isopropyl alcohol to achieve cleanliness levels corresponding to level 200 for particulates and 20 ng cm⁻² for molecular film residues. Class 10 000, or better, air quality was maintained when sampling system hardware that was exposed for integration and testing. Surfaces of the sampling system were assessed for contaminants using swabs and high purity hexane. The redistribution of particulate and molecular materials, e.g., by shock and acoustic excitation, during the launch, cruise, entry, descent, and landing events was recognized to provide a means for contaminant transport. During the mission cruise phase, electronic units were maintained under vacuum with elevated temperature leading to outgassing and condensation of volatile contaminants elsewhere in the system.

One method by which terrestrial organic contamination may be further reduced is by dilution cleaning (Anderson *et al.*, 2012). In the context of Mars, dilution cleaning involves the use of Mars regolith to repeatedly contact hardware surfaces to physically remove contaminants from surfaces within the sample transfer path.

WHAT ARE THE STANDARD PLANETARY PROTECTION STRATEGIES CURRENTLY USED FOR ICY MOONS?

The Planetary Protection Categories defined by the Committee on Space Research (COSPAR) differentiate between space missions according to their type (flyby, orbiter, lander, sample return), while also taking into account the degree to which the contaminants of a spacecraft might compromise the processes of understanding local chemical evolution and/or the origin of life. COSPAR’s Panel on Planetary Protection (Kminek and Rummel, 2015; Rummel *et al.*, 2010) provided an extended, but simplified, version of a procedure that had

previously been recommended by the National Council for Research. This divides the icy bodies of the outer solar system into three groups for analysis: (1) A large group of objects, including small icy bodies, which were judged to have only a ‘remote’ chance of contamination by spacecraft missions of all types. (2) A group consisting of Ganymede, Titan, Triton, Pluto/Charon and those Kuiper belt objects with diameters greater than one half that of Pluto, that were also thought to pose a ‘remote’ concern for contamination. (3) A group/pair consisting of Europa and Enceladus that were believed to have a ‘significant’ chance of contamination by spacecraft missions. The ‘significant’ chance of contamination referred to in (3) requires the implementation of significant measures (potentially including bioburden reduction), for flybys as well as for orbiter and lander missions to Europa and Enceladus, aimed overall at reducing the probability of inadvertent contamination of bodies of water beneath the surfaces of these objects to $<1 \times 10^{-4}$ per mission.

The approach adopted by COSPAR for determining compliance with its 10^{-4} standard for missions targeted to Europa (and Enceladus), and to a lesser extent for missions to Ganymede (including also Titan, Triton, Pluto/Charon and large Kuiper belt objects), requires the addressing of conservatively estimated, although as yet poorly known, parameters. In this regard, in the case of Europa, the following items are presently included (at a minimum) in the probability estimation. i) Bioburden at launch, ii) Cruise survival of contaminating organisms, iii) Organism survival in the environment of, and on, Europa, iii) Probability of landing on Europa, iv) The mechanisms and timescales of transport to the subsurface of Europa and v) Organism survival and proliferation before, during and after subsurface transfer. This particular approach also leaves open the possibility to include additional parameters in the calculation.

WHAT EXISTING PLANETARY PROTECTION TECHNIQUES ARE

PARTICULARLY USEFUL FOR ICY MOONS?

The Task Group on the Forward Contamination of Europa concluded that current cleaning and sterilization techniques are sufficient to meet the needs of future space missions to Europa. Some useful definitions are listed below (Chosewood and Wilson 2009):

- Sterilization: processes aimed at complete elimination (through removal, inactivation or killing) of all living microorganisms and viruses (see Glossary).
- Disinfection: elimination of nearly all recognized pathogenic microorganisms but not necessarily all microbial forms.
- Decontamination: Ensuring that an item is safe to handle and reasonably free from the transmission of organisms, including removal of contaminating agents, e.g., microorganisms or organic compounds and potentially of biomatter from dead microorganisms (see Glossary)

These techniques include Viking-derived procedures such as cleaning surfaces with isopropyl alcohol and/or sporicides and sterilization by dry heating, as well as more modern processes such as sterilization by hydrogen peroxide, demonstration by analysis that adequate sterilization is accomplished via exposure of the spacecraft to Europa’s radiation environment (NRC, 2006). A significant challenge is the need to sterilize the encapsulated spacecraft bioburden. This will require extended exposure to sterilizing dry-heat temperatures (dry heat is one of only two technologies that can effectively sterilize an encapsulated bioburden, the other being a penetrating ionizing radiation). Therefore, spacecraft material must be carefully selected to be compatible with the selected mode of sterilization. All components of the probe instrumentation must be decontaminated for chemical and biological reduction using both physical and chemical treatments depending on the material properties and size of the component part.

In brief, physical methods could include dry heat up to 250°C (for bulky heat-resistant parts), steam at 121°C (for surfaces of heat and

water-resistant components), germicidal UV radiation at 254 nm (for all UV-resistant and unshadowed surfaces) and ionizing radiation (Frick *et al.*, 2014; Konstantinidis *et al.*, 2015). Chemical methods for cleaning, bioburden reduction and/or sterilization could include detergent alkaline mixtures, alcohol mixtures, hydrogen peroxide ($\geq 5\%$), hypochlorous acid (for stainless steel parts), supercritical carbon dioxide, ethylene oxide and ozone (Frick *et al.*, 2014; Konstantinidis *et al.*, 2015). For each component, a combination of decontamination methods leads to greater effectiveness. Attention needs to be paid to matching process parameters with the hardware for the mission. For example, heat eventually kills all microbes (5 hours at 125°C reduce surface numbers by many orders of magnitude). Electronic components utilized onboard a mission to the icy moons should thus be suitably qualified to withstand processes aimed at reducing the overall contamination.

WHAT NEW PLANETARY PROTECTION TECHNIQUES NEED TO BE DEVELOPED FOR ICY MOONS?

Owing to the great distances of the icy moons from the Sun, nuclear power sources may be adopted for transport. Current planetary protection rules concerning nuclear power sources on celestial bodies are not explicit. However, it can be assumed that there will be no desire to introduce perennial heat sources under the ice together with contaminated spacecraft hardware. To meet the probabilistic requirements of radioactive material coming into contact with the ocean present on an icy moon it is anticipated that strict limits will be imposed (Konstantinidis *et al.*, 2015). It is likely that planetary protection requirements will in future require careful consideration in that regard. The potential global connectivity of subsurface oceans means that microbial contamination could spread across the whole moon (NRC 2006). It has thus been recommended that current Mars-focused spore-based culturing techniques used to estimate the total bio-load on a spacecraft should be supplemented

by screening tests for specific types of extremophiles, such as radiation-resistant organisms (NRC, 2006). It has also been suggested that modern molecular methods, such as those based on the polymerase chain reaction (PCR), may prove to be more rapid and more informative for detecting and identifying biological contamination than NASA's and ESA's existing culturing protocols for planetary protection (NRC, 2006).

WHAT ORGANIC CLEANLINESS CONSIDERATIONS ARE PARTICULARLY USEFUL FOR ICY MOONS?

In the case of icy moons, the organic cleaning steps used for Mars during hardware assembly are directly transferable. Perhaps the most significant difference between icy moons and Mars is the type of sample available for analysis. Mars samples of regolith or rock are encountered as, or are transformed to, granular materials. When orbiting, icy moons, samples may be present as volatile or particulate plumes. For example, Enceladus is venting plumes of water ice, methane, and simple organic compounds (Postberg *et al.*, 2009) and transient plumes have also been suggested for Europa (Roth *et al.*, 2014, Sparks *et al.*, 2016). When landed, icy moon sampling missions will encounter icy solids or possibly liquid water. The dilution cleaning approach utilized for Mars may be even more relevant for landed operations because of the ease with which water dissolves organic contamination, but only if possibilities for forward contamination of the moons and their astrobiologically-relevant environments are adequately addressed.

WHAT MISSIONS ARE BEING PLANNED FOR THE ICY MOONS?

There are a number of forthcoming missions to the icy moons that will require planetary protection and organic contamination considerations. Preparations for the ESA Jupiter Icy Moons Explorer (JUICE) mission are now at an advanced stage and it will involve a flyby of Jupiter and its icy moons Ganymede, Callisto and Europa. Part of its objectives will be to search for organic compounds (Grasset *et al.*,

2013). The NASA Europa Clipper mission will repeatedly flyby Jupiter's icy moon, passing 25-100 km above the surface. The Europa Clipper mission will: seek evidence for liquid water in the subsurface, determine the thickness of the ice crust, obtain evidence of material exchange and the geomorphology of the surface and perform mass spectrometric detection of plume materials for evidence of habitability (Phillips and Pappalardo, 2014).

CONCLUSIONS

Our understanding of the transfer and survival of terrestrial microorganism and the development of planetary protection protocols to prevent their introduction to inner solar system bodies such as Mars provides a strong foundation for the development of similar protocols for the outer solar system. Our existing knowledge must be adapted and enhanced because the icy moons of the outer solar system provide distinct challenges for planetary protection. The potential presence of abundant and contiguous liquid water, organic compounds, intense radiation and tidal energy-driven rock-water interactions provide the possibility of efficient proliferation following contamination from Earth. In addition to planetary protection, organic cleanliness is also an essential requirement for missions to the outer solar system. The potentially habitable environments on icy moons that may be colonized by terrestrial microorganisms also hold the possibility of hosting indigenous biospheres. Organic contamination by Earth-sourced materials would confuse or corrupt indigenous signals of life. Forthcoming missions, such as ESA's JUICE and NASA's Europa Clipper, will benefit from new planetary protection and organic cleanliness protocols that protect the outer solar system environment and maximize the scientific return from any data acquired.

Anderson M.S., Katz I., Petkov M., Blakkolb B., Mennella J., D'Agostino S., Crisp J., Evans J., Feldman J., and Limonadi D. (2012): In situ cleaning of instruments for the sensitive detection of organics on Mars. *Review of Scientific Instruments*, 83, 105109.

Bagenal F., Sidrow E., Wilson R.J., Cassidy T.A., Dols V., Crary F.J., Steffl A.J., Delamere P.A., Kurth W.S., and Paterson W.R. (2015): Plasma conditions at Europa's orbit. *Icarus*, 261, 1-13.

Blakkolb B., Logan C., Jandura L., Okon A., Anderson M., Katz I., Aveni G., Brown K., Chung S., Ferraro N. and others. (2014): Organic cleanliness of the Mars Science Laboratory sample transfer chain. *Rev. Sci. Instrum.*, 85, 075111.

Cantor B.A., James P.B., Caplinger M., and Wolff M.J. (2001): Martian dust storms: 1999 Mars Orbiter Camera observations. *Journal of Geophysical Research*, 106, 23653-23688.

Cassidy T., Coll P., Raulin F., Carlson R.W., Johnson R.E., Loeffler M.J., Hand K.P., and Baragiola R.A. (2010): Radiolysis and Photolysis of Icy Satellite Surfaces: Experiments and Theory. *Space Science Reviews*, 153, 299-315.

Chosewood L.C., and Wilson D.E. (2009): Biosafety in Microbiological and Biomedical Laboratories U.S. Department of Health and Human Services, Washington D.C.

Cockell C.S., and Barlow N.G. (2002): Impact excavation and the search for subsurface life on Mars. *Icarus*, 155, 340-349.

Court R.W., Sephton M.A., Parnell J., and Gilmore I. (2006): The alteration of organic matter in response to ionising irradiation: Chemical trends and implications for extraterrestrial sample analysis. *Geochimica et Cosmochimica Acta*, 70, 1020-1039.

ESA (2016): Schiaparelli landing investigation makes progress.

Frick A., Mogul R., Stabekis P., Conley C.A., and Ehrenfreund P. (2014): Overview of current capabilities and research and technology developments for planetary protection. *Advances in Space Research*, 54, 221-240.

Grotzinger J.P., Crisp J., Vasavada A.R., Anderson R.C., Baker C.J., Barry R., Blake D.F., Conrad P., Edgett K.S., Ferdowski B. and others (2012): Mars Science Laboratory Mission and Science Investigation. *Space Science Reviews*, 170, 5-56.

- Hand K.P., Carlson R.W., and Chyba C.F. (2007): Energy, Chemical Disequilibrium, and Geological Constraints on Europa. *Astrobiology*, 7, 1006-1022.
- Hansen C.J., Esposito L., Stewart A.I.F., Colwell J., Hendrix A., Pryor W., Shemansky D., and West R. (2006): Enceladus' Water Vapor Plume. *Science*, 311, 1422-1425.
- He C., and Smith M.A. (2014): Identification of nitrogenous organic species in Titan aerosols analogs: Implication for prebiotic chemistry on Titan and early Earth. *Icarus*, 238, 86-92.
- Hsu H.-W., Postberg F., Sekine Y., Shibuya T., Kempf S., Horanyi M., Juhasz A., Altobelli N., Suzuki K., Masaki Y. and others (2015): Ongoing hydrothermal activities within Enceladus. *Nature*, 519, 207-210.
- ISO (2016): ISO 14644-1:2015 - Cleanrooms and associated controlled environments - Part 1: Classification of air cleanliness by particle concentration.
- Kargel J.S., Kaye J.Z., Head J.W., Marion G.M., Sassen R., Crowley J.K., Ballesteros O.P., Grant S.A., and Hogenboom D.L. (2000): Europa's Crust and Ocean: Origin, Composition, and the Prospects for Life. *Icarus*, 148, 226-265.
- Kimura J., and Kitadai N. (2015): Polymerization of Building Blocks of Life on Europa and Other Icy Moons. *Astrobiology*, 15, 430-441.
- Kivelson M.G., Khurana K.K., Russell C.T., Volwerk M., Walker R.J., and Zimmer C. (2000): Galileo Magnetometer Measurements: A Stronger Case for a Subsurface Ocean at Europa. *Science*, 289, 1340-1343.
- Kivelson M.G., Khurana K.K., and Volwerk M. (2002): The Permanent and Inductive Magnetic Moments of Ganymede. *Icarus*, 157, 507-522.
- Klein H.P. (1976): The Viking Mission search for life on Mars. *Nature*, 262, 24-27.
- Klein H.P. (1978): The Viking biological experiments on Mars. *Icarus*, 34, 666-674.
- Kminek G, and Bada J.L. (2006): The effect of ionizing radiation on the preservation of amino acids on Mars. *Earth and Planetary Science Letters*, 245, 1-5.
- Kminek G., and Rummel J.D. (2015): CO-SPAR's Planetary Protection Policy. *Space Research Today*, 193, 7-19.
- Konstantinidis K., Flores Martinez C.L., Dachwald B., Ohndorf A., Dykta P., Bowitz P., Rudolph M., Digel I., Kowalski J., Voigt K. and others (2015): A lander mission to probe subglacial water on Saturn's moon Enceladus for life. *Acta Astronautica*, 106, 63-89.
- Leshin L.A., Mahaffy P.R., Webster C.R., Cabane M., Coll P., Conrad P.G., Archer P.D., Atreya S.K., Brunner A.E., Buch A. and others (2013): Volatile, isotope, and organic analysis of Martian fines with the Mars Curiosity Rover. *Science*, 341, 1238937.
- Levin G.V., and Straat P.A. (1976): Viking labeled release biology experiment - Interim results. *Science*, 194, 1322-1329.
- Levin G.V., and Straat P.A. (1979): Viking Mars labelled release results. *Nature*, 277, 326.
- Mahaffy P.R., Beaty D.W., Anderson M.S., Aveni G., Bada J.L., Clemett S.J., Des Marais D.J., Douglas S., Dworkin J.P., Kern R.G. and others (2004): Report of the Organic Contamination Science Steering Group. In: *Lunar and Planetary Science Conference*. edited by S Mackwells.
- McCullom T.M., Ritter G., and Simoneit B.R. (1999): Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. *Orig. Life Evol. Biosph.*, 29, 153-66.
- McKay C.P., Anbar A.D., Porco C., and Tsou P. (2014): Follow the plume: the habitability of Enceladus. *Astrobiology*, 14, 352-5.
- Ming D.W., Archer P.D., Glavin D.P., Eigenbrode J.L., Franz H.B., Sutter B., Brunner A.E., Stern J.C., Freissinet C., McAdam A.C. and others (2014): Volatile and organic compositions of sedimentary rocks in Yellowknife Bay, Gale Crater, Mars. *Science*, 343, 1245267.

- Neish C.D., Somogyi Á., and Smith M.A. (2010): Titan's Primordial Soup: Formation of Amino Acids via Low-Temperature Hydrolysis of Tholins. *Astrobiology*, 10, 337-347.
- NRC (2006): National Research Council Report on Preventing the forward contamination of Mars. National Academies Press, Washington DC.
- Pascal R. (2016): Physicochemical Requirements Inferred for Chemical Self-Organization Hardly Support an Emergence of Life in the Deep Oceans of Icy Moons. *Astrobiology*, 16, 328-34.
- Pavlov A.A., Vasilyev G., Ostryakov V.M., Pavlov A.K., and Mahaffy P. (2012) Degradation of the organic molecules in the shallow subsurface of Mars due to irradiation by cosmic rays. *Geophysical Research Letters*, 39, L13202.
- Phillips C.B., McEwen A.S., Hoppa G.V., Fagents S.A., Greeley R., Klemaszewski J.E., Pappalardo R.T., Klaasen K.P., and Breneman H.H. (2000): The search for current geologic activity on Europa. *Journal of Geophysical Research*, 105, 22579-22598.
- Porco C.C., Helfenstein P., Thomas P.C., Ingersoll A.P., Wisdom J., West R., Neukum G., Denk T., Wagner R., Roatsch T. and others (2006): Cassini Observes the Active South Pole of Enceladus. *Science*, 311, 1393-1401.
- Raulin F., Hand K.P., McKay C.P., and Viso M. (2010): Exobiology and Planetary Protection of icy moons. *Space Science Reviews*, 153, 511-535.
- Rettberg P., Anesio A., Baker V., Baross J., Cady S., Foreman C., Hauber E., Ori G., Pearce D., Renno N. and others (2015): Review of the MEPAG Report on Mars Special Regions. The National Academies Press, Washington, DC.
- Rettberg P., Anesio A.M., Baker V.R., Baross J.A., Cady S.L., Detsis E., Foreman C.M., Hauber E., Ori G.G., Pearce D.A. and others (2016): Planetary Protection and Mars Special Regions—A Suggestion for Updating the Definition. *Astrobiology*, 16, 119-125.
- Rummel J.D., Ehrenfreund P., and Peter N. (2010): COSPAR Workshop on Planetary Protection for Outer Planet Satellites and Small Solar System Bodies. In: *38th COSPAR Scientific Assembly*, p 4.
- Rummel J.D., Beaty D.W., Jones M.A., Bakermans C., Barlow N.G., Boston P.J., Chevrier V.F., Clark B.C., de Vera J.-P.P., Gough R.V. and others (2014): A New Analysis of Mars “Special Regions”: Findings of the Second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology*, 14, 887-968.
- Schuerger A.C., Mancinelli R.L., Kern R.G., Rothschild L.J., and McKay C.P. (2003): Survival of endospores of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: implications for the forward contamination of Mars. *Icarus*, 165, 253-276.
- Sephton M.A. (2004): Origin of Life. In: *An Introduction to Astrobiology*. Edited by I. Gilmour and M.A. Sephton, Cambridge University Press, Cambridge, p 1-41.
- Sleep N.H. (1994): Martian plate tectonics. *Journal of Geophysical Research*, 99, 5.
- Spencer J.R., and Nimmo F. (2013): Enceladus: An Active Ice World in the Saturn System. *Annual Review of Earth and Planetary Sciences*, 41, 693-717.
- Tauscher C., Schuerger A.C., and Nicholson W.L. (2006): Survival and Germinability of *Bacillus subtilis* Spores Exposed to Simulated Mars Solar Radiation: Implications for Life Detection and Planetary Protection. *Astrobiology*, 6, 592-605.
- Vaishampayan P.A., Fox G.E., and Venkateswaran K. (2010): Survival of *Bacillus pumilus* SAFR-032 in simulated Mars atmosphere in real space conditions. In: *Instruments, Methods, and Missions for Astrobiology XIII*.
- Vance S., Harnmeijer J., Kimura J., Hussmann H., deMartin B., and Brown J.M. (2007): Hydrothermal systems in small ocean planets. *Astrobiology*, 7, 987-1005.
- Waite Jr J.H., Lewis W.S., Magee B.A., Lunine J.I., McKinnon W.B., Glein C.R., Mousis O., Young D.T., Brockwell T., Westlake J. and

others (2009): Liquid water on Enceladus from observations of ammonia and ^{40}Ar in the plume. *Nature*, 460, 487-490.

West R., Neukum G., Denk T., Wagner R., Roatsch T. and others (2006): Cassini Observes the Active South Pole of Enceladus. *Science*, 311, 1393-1401.

Zolotov M.Y. (2007): An oceanic composition on early and today's Enceladus. *Geophysical Research Letters*, 34.

Zolotov M.Y., and Shock E.L. (2001): Composition and stability of salts on the surface of Europa and their oceanic origin. *Journal of Geophysical Research*, 106, 32815-32828.

Handbook Objectives

28

Understanding potential hazards is essential to pursue Space Exploration. Avoiding unnecessary challenges coming from introducing unwanted forward invasive Earth contamination while searching for extra-terrestrial life is key to efficiently pursuing exploration. The Handbook describes the state-of-the-art and good-practices to implement planetary protection requirements internationally.

This handbook will help you:

- Understand what planetary protection is, and why it is important;
- Get acquainted with the Outer Space Treaty and the ESA/NASA requirements and their implementation;
- Learn about the state of the art regarding Best Practice of Organic Contamination

Control (BPOCC) for Icy Moons and Mars missions;

- Discover the lessons learned from Planetary Protection experts, underlining the dos and don'ts in Planetary Protection implementation; and,
- Familiarize yourself with Planetary Protection implementation through the Planetary Protection Check List.

To that end, this Handbook will go through the basics of microbiology needed to understand Planetary Protection processes. It will then go through Planetary Protection at ESA and NASA, the best practices in organic contamination control, the practical lessons learned through Q&A and the Planetary Protection Check-list.

International Partners

This handbook has been coordinated with international partners from ESA, NASA, CNES, CAST, and the JAXA planetary protection office to ensure that requirements, documentation and reviews cover the needs and obligations of the respective space agencies for joint missions.

Four training workshops¹ (“Tutorials”) were held using an initial version of this Handbook, in Tsukuba, Japan (May 2017), Pasadena, CA, USA (July 2018), Bremen, Germany (October 2018) and Beijing, China (October 2018).

¹ Visit www.pposs.org/tutorial/

Chapter 1. Planetary Protection Basics

The present chapter provides an overview of planetary protection basics, including an introduction to the COSPAR Panel on Planetary Protection, elements of microbiology, basic information on bioburden, assay and sterilization and some useful rudiments of orbital dynamics.

PLANETARY PROTECTION BASICS²

WHAT PLANETARY PROTECTION IS NOT

To begin with, a first clarification of what planetary protection *is not* may be useful:

- It is not about asteroid defense:
 - Covered in the Near Earth Objects (NEO) and Space Situational Awareness (SSA) programs.
- It is not about space debris:
 - Covered in the Space Surveillance and Tracking (SST), space debris, and sustainability programs.
- It is not about cultural or natural world heritage:
 - Covered by UNESCO based on a convention (for Earth) and the COSPAR Panel on Exploration (for space).
- It is not a green party for space.
- It is not about playing around with guns and ET.

HISTORY OF PLANETARY PROTECTION

“...we are in the awkward situation of being able to spoil certain possibilities for scientific investigations for a considerable interval before we can constructively realize them...we urgently need to give some thought to the conservative measures needed to protect future scientific objectives on the moon and the planets...” (J. Lederberg and D. B. Cowie, *Science*, 1958).

The above statement reflects the concern raised by the International Astronautical Fed-

eration (IAF), UN-COPUOS and the US National Academy of Science (NAS) in this time period that led to the Committee on Contamination by Extraterrestrial Exploration (CETEX), established by the International Council of Scientific Unions (ICSU).

In 1958, ICSU adopted the CETEX Code-of-Conduct and established the Committee on Space Research (COSPAR).

COSPAR established the Consultative Group on Potentially Harmful Effects of Space Experiments.

The first spaceflight missions to use this Code-of-Conduct were the Ranger missions in 1961. Since then, all planetary missions had to implement planetary protection measures at different degrees – ranging from simple documentation to terminal sterilization of entire flight systems.

More detailed quantitative regulations, in particular for Mars, were adopted by COSPAR in 1964 (e.g., C. Sagan and S. Coleman, *Astrophysics & Aeronautics*, 1965; C. Sagan, E. C. Levinthal, J. Lederberg, *Science*, 1968).

RATIONALE FOR PLANETARY PROTECTION

The rationale for planetary protection can be formulated as follows:

- **Ensure that scientific investigations related to the origin and distribution of life are not compromised:**
 - Protecting our investment in space science and exploration;
 - Protect the unique opportunity to learn more about the origin of life in a way that is no longer possible on Earth because of reprocessing/overprinting of

² This section is based on a presentation by Dr. Gerhard Kminek.

earliest evidences of life by the abundant more recent life forms;

- and then, there is the more philosophical issue about the Drake equation.
- **Protect the Earth from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from an interplanetary mission:**
 - Simple prudence – protect the Earth!
 - In line with the precautionary principle of environmental protection.

FRAMEWORK FOR PLANETARY PROTECTION

The legal basis and the goal for planetary protection were established in Article IX of 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 (UN Outer Space Treaty, 1967):

“...parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose...”

COSPAR maintains and promulgates a planetary protection policy for the reference of spacefaring nations, both as an international standard on procedures to avoid organic constituent and biological contamination in space exploration, and to provide accepted guidelines and requirements in this area to guide compliance with the wording of the Outer Space Treaty.

PLANETARY PROTECTION CATEGORIES

The different planetary protection categories (I-V) reflect the level of interest and concern

that contamination can compromise future investigations; the categories and associated requirements depend on target body and mission type combinations.

Category I: All types of mission to a target body which is not of direct interest for understanding the process of chemical evolution or the origin of life.

Category II: All types of missions (gravity assist, orbiter, lander) to a target body where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote³ chance that contamination carried by a spacecraft could compromise future investigations.

Category III: Flyby (i.e., gravity assist) and orbiter missions to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant⁴ chance of contamination which could compromise future investigations.

Category IV: Lander/impactor missions to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant⁵ chance of contamination which could compromise future investigations.

Category V: Two subcategories exist - unrestricted Earth return for solar system bodies deemed by scientific opinion to have no indigenous life forms, and restricted Earth return for all others.

The following list summarizes the Planetary Protection Categories in terms of type of missions and targets:

Category I: Flyby, Orbiter, Lander: Undifferentiated, metamorphosed asteroids; others TBD.

Category II: Flyby, Orbiter, Lander: Venus; Moon (with organic inventory); Comets; Car-

³ Implies the absence of environments where terrestrial organisms could survive and replicate, or a very low likelihood of transfer to environments where terrestrial organisms could survive and replicate.

⁴ Implies the presence of environments where terrestrial organisms could survive and replicate, and some likelihood of transfer to those places by a plausible mechanism

⁵ Same as above note.

bonaceous Chondrite Asteroids; Jupiter; Saturn; Uranus; Neptune; Ganymede†; Titan†; Triton†; Pluto/Charon†; Ceres; Kuiper-Belt Objects > 1/2 the size of Pluto†; Kuiper-Belt Objects < 1/2 the size of Pluto; others TBD.

Category III: Flyby, Orbiters: Mars; Europa; Enceladus; others TBD.

Category IV: Lander Missions: Mars; Europa; Enceladus; others TBD.

Category V: Any Earth-return mission.

- “Restricted Earth return”: Mars; Europa; Enceladus; others TBD.
- “Unrestricted Earth return”: Venus, Moon; others TBD.

This list is current at the time of writing, but is periodically updated based on latest scientific information.

† denotes the need for additional analysis.

PLANETARY PROTECTION REQUIREMENTS

The Planetary Protection requirements are described hereafter in more detail.

MARS

Launcher upper stage⁶: The probability of impact on Mars by any element not assembled and maintained in ISO level 8 conditions shall be $\leq 1 \times 10^{-4}$ for the first 50 years after launch.

Category III: gravity assist, orbiter, orbiter/lander composite, cruise stage/lander composite⁷. One of the following conditions shall be met:

- The probability of impact on Mars by any part of a spacecraft assembled and maintained in ISO level 8 cleanrooms, or better, is $\leq 1 \times 10^{-2}$ for the first 20 years after launch, and $\leq 5 \times 10^{-2}$ for the time period from 20 to 50 years after launch;
- The total bioburden of the spacecraft, including surface, mated, and encapsulated bioburden, is $\leq 5 \times 10^5$ bacterial spores.

Category IV: All the requirements for category III as well as:

Category IVa: Lander systems not carrying instruments for the investigations of extant Martian life are restricted to a surface bioburden level of $\leq 3 \times 10^5$ spores, and an average of ≤ 300 spores per square meter.

Category IVb: For lander systems designed to investigate extant Martian life, all of the requirements of Category IVa apply, along with the following requirement:

- The entire landed system is restricted to a surface bioburden level of ≤ 30 spores, or to levels of bioburden reduction driven by the nature and sensitivity of the particular life-detection experiments,

OR

- The subsystems which are involved in the acquisition, delivery, and analysis of samples used for life detection must be sterilized to these levels, and a method of preventing recontamination of the sterilized subsystems and the contamination of the material to be analyzed is in place.

Category IVc: For missions which investigate Martian special regions (see definition below), even if they do not include life detection experiments, all of the requirements of Category IVa apply, along with the following requirement:

- Case 1. If the landing site is within the special region, the entire landed system is restricted to a surface bioburden level of ≤ 30 spores
- Case 2. If the special region is accessed through horizontal or vertical mobility, either the entire landed system is restricted to a surface bioburden level of ≤ 30 spores,

OR

- the subsystems which directly contact the special region shall be sterilized to these

⁶ Based on ESA ESSB-ST-U-001, editorial modification of COSPAR text.

⁷ Same as above note.

levels, and a method of preventing their re-contamination prior to accessing the special region shall be provided.

If an off-nominal condition (such as a hard landing) would cause a high probability of inadvertent biological contamination of the special region by the spacecraft, the entire landed system must be sterilized to a surface bioburden level of ≤ 30 spores and a total (surface, mated, and encapsulated) bioburden level of $\leq 30 + (2 \times 10^5)$ spores.

Planned 3-sigma pre-launch landing ellipses must be evaluated on a case-by-case basis as part of the (landing) site selection process, to determine whether the mission would land or come within contamination range of areas or volumes meeting the parameter definition for Mars Special Regions or would impinge on already described features that must be treated as Mars Special Regions.

The evaluation must be based on the latest scientific evidence and in particular include an assessment of the extent to which the temperature and water activity values specified for Mars Special Regions are separated in time. The evaluation must be updated during the mission whenever new evidence indicates that the landing ellipse and/or the operational environment contain or are in contamination range of areas or volumes meeting the parameter definition for Mars Special Regions or already described features that must be treated as Mars Special Regions.

Definition of Special Region

A Special Region is defined as a region within which terrestrial organisms are likely to replicate. Any region which is interpreted to have a high potential for the existence of extant Martian life forms is also defined as a Special Region. Given current understanding of terrestrial

organisms, Special Regions are defined as areas or volumes within which sufficient water activity AND sufficiently warm temperatures to permit replication of Earth organisms may exist.

The physical parameters delineating applicable water activity and temperature thresholds are given below:

- Lower limit for water activity: 0.5; Upper limit: 1.0;
- Lower limit for temperature: -28°C ; No Upper limit defined;

The timescale within which limits can be identified is 500 years.

Observed features to be treated as Special Regions until demonstrated otherwise:

- Gullies (taxon 2-4)⁸, and bright streaks associated with gullies;
- Subsurface cavities;
- Subsurface below 5 meters;
- Confirmed and partially confirmed Recurrent Slope Lineae (RSL)⁹.

Features, if found, to be treated as a Special Region until demonstrated otherwise:

- Groundwater;
- Source of methane;
- Geothermal activity;
- Modern outflow channel.

Observed features that require a **case-by-case** evaluation before being classified as a Special Region:

- Dark streaks;
- Pasted-on terrain;
- Candidate RSL¹⁰.

Spacecraft-induced special regions are to be evaluated, consistent with these limits and features, on a case-by-case basis.

⁸ A description for Gully taxon can be found in Rummel *et al.*, 2014.

⁹ Observational evidence for Recurrent Slope Lineae (RSL), adapted McEwen *et al.*, 2014:

- Confirmed: observed simultaneous incremental growth of flows on a warm slope, fading, and recurrence of this sequence in multiple Mars years;

- Partially confirmed: observed either incremental growth or recurrence;

- Candidate: slope lineae that resemble RSL but where observations needed for partial confirmation are currently lacking.

¹⁰ Same as above note.

In the absence of specific information, no Special Regions are currently identified on the basis of possible Martian life forms.

Category V: Earth return missions from Mars are classified, “Restricted Earth return”

- Unless specifically exempted, the outbound leg of the mission shall meet Category IVb requirements;
- Unless the samples to be returned from Mars are subjected to an accepted and approved sterilization process, the canister(s) holding the samples returned from Mars shall be closed, with an appropriate verification process, and the samples shall remain contained during all mission phases through transport to a receiving facility where it (they) can be opened under containment;
- The mission and the spacecraft design must provide a method to “break the chain of contact” with Mars, i.e., no uncontained hardware that contacted Mars, directly or indirectly, shall be returned to Earth;
- Reviews and approval of the continuation of the flight mission shall be required at three stages: 1) prior to launch from Earth; 2) prior to leaving Mars for return to Earth; and 3) prior to commitment to Earth reentry;
- For unsterilized samples returned to Earth, a program of life detection and biohazard testing, or a proven sterilization process, shall be undertaken as an absolute precondition for the controlled distribution of any portion of the sample.

Principles for Human Missions to Mars

The intent of the COSPAR planetary protection policy is the same whether a mission to Mars is conducted robotically or with human explorers;

- Planetary protection goals should not be relaxed to accommodate a human mission to Mars, i.e., they become even more directly relevant to such missions – even if specific implementation requirements must differ;

- Safeguarding the Earth from potential back contamination is the highest planetary protection priority in Mars exploration;
- The greater capability of human explorers can contribute to the astrobiological exploration of Mars only if human-associated contamination is controlled and understood.

Establishment of engineering requirements responsive to these principles is under way through a series of NASA and COSPAR workshops involving all the necessary stakeholders.

EUROPA AND ENCELADUS

Category III and IV: Requirements for Europa and Enceladus flybys, orbiters and landers, including bioburden reduction, shall be applied in order to reduce the probability of inadvertent contamination of a European or Enceladian ocean to less than 1×10^{-4} per mission.

The probability of inadvertent contamination of a European or Enceladian ocean of 1×10^{-4} applies to all mission phases including the duration beyond end-of-mission that spacecraft introduced terrestrial organisms remain viable and could reach a subsurface liquid water environment.

Specific requirements will likely be refined and standardized in future years, but the calculation of this probability should include a conservative estimate of poorly known parameters, and address the following factors, at a minimum:

- Bioburden at launch;
- Cruise survival for contaminating organisms;
- Organism survival in the radiation environment adjacent to Europa or Enceladus;
- Probability of landing on Europa or Enceladus;
- The mechanisms and timescales of transport to a European or Enceladian subsurface liquid water environment;
- Organism survival and proliferation before, during, and after subsurface transfer.

Preliminary calculations of the probability of contamination suggest that **bioburden reduction** will likely be necessary even for Europa and Enceladus **orbiters** (Category III) as well as for **landers**, requiring the use of cleanroom technology and the cleanliness of all parts before assembly, and the monitoring of spacecraft assembly facilities to understand the bioburden and its microbial diversity, including specific problematic species.

Methods of **bioburden reduction** should reflect the type of environments found on Europa or Enceladus, focusing on **Earth extremophiles** most likely to survive on Europa or Enceladus, such as cold and radiation tolerant organisms.

Category V: Earth return missions from Europa and Enceladus are classified, “Restricted Earth return”:

- Unless specifically exempted, the outbound leg of the mission shall meet requirements for life detection missions;
- Unless the samples to be returned from Europa or Enceladus are subjected to an accepted and approved sterilization process, the canister(s) holding the samples returned from Europa or Enceladus shall be closed, with an appropriate verification process, and the samples shall remain contained during all mission phases through transport to a receiving facility where it (they) can be opened under containment;
- The mission and the spacecraft design must provide a method to “break the chain of contact” with Europa or Enceladus, i.e., no uncontained hardware that contacts material from Europa, Enceladus or their plumes, shall be returned to the Earth’s biosphere or the Moon;
- Reviews and approval of the continuation of the flight mission shall be required at three stages: 1) prior to launch from Earth; 2) subsequent to sample collection and prior to a maneuver to enter a biased Earth return trajectory; and 3) prior to commitment to Earth re-entry;
- For unsterilized samples returned to Earth, a program of life detection and biohazard

testing, or a proven sterilization process, shall be undertaken as an absolute precondition for the controlled distribution of any portion of the sample.

SMALL SOLAR SYSTEM BODIES

The small bodies of the solar system not elsewhere discussed in this policy represent a very large class of objects.

Imposing forward contamination controls on these missions is not warranted except on a case-by-case basis, so most such missions should reflect Categories I or II.

Category V: Determination as to whether a mission is classified “Restricted Earth return” or not shall be undertaken with respect to the best multidisciplinary scientific advice.

Specifically, such a determination shall address the following six questions for each body intended to be sampled:

1. Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?
2. Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?
3. Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO₂ or carbonates and an appropriate source of reducing equivalents) in or on the target body to support life?
4. Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., > 160°C)?
5. Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life forms?
6. Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

For containment procedures to be necessary (“Restricted Earth return”), an answer of “no” or “uncertain” needs to be returned to all six questions.

For missions determined to be Category V, “Restricted Earth return,” the following requirements shall be met:

- Unless specifically exempted, the outbound leg of the mission shall meet requirements for life detection missions;
- Unless the samples to be returned are subjected to an accepted and approved sterilization process, the canister(s) holding the samples shall be closed, with an appropriate verification process, and the samples shall remain contained during all mission phases through transport to a receiving facility where it (they) can be opened under containment;
- The mission and the spacecraft design must provide a method to “break the chain of contact” with the small body, i.e., no uncontained hardware that contacted the body, directly or indirectly, shall be returned to Earth;
- Reviews and approval of the continuation of the flight mission shall be required at three stages: 1) prior to launch from Earth; 2) prior to leaving the body or its environment for return to Earth; and 3) prior to commitment to Earth re-entry;
- For unsterilized samples returned to Earth, a program of life detection and biohazard testing, or a proven sterilization process, shall be undertaken as an absolute precondition for the controlled distribution of any portion of the sample.

REPORTING TO COSPAR

COSPAR,

Recommends that COSPAR members inform COSPAR when establishing planetary protection requirements for planetary missions, and

Recommends that COSPAR members provide information to COSPAR within a reasonable time not to exceed six months after launch about the procedures and computations used for planetary protection for each flight and

again within one year after the end of a solar-system exploration mission about the areas of the target(s) which may have been subject to contamination. COSPAR will maintain a repository of these reports, make them available to the public, and annually deliver a record of these reports to the Secretary General of the United Nations. For multinational missions, it is suggested that the lead partner should take the lead in submitting these reports.

Reports should include, but not be limited to, the following information:

1. The estimated bioburden at launch, the methods used to obtain the estimate (e.g., assay techniques applied to spacecraft or a proxy), and the statistical uncertainty in the estimate;
2. The probable composition (identification) of the bioburden for Category IV missions, and for Category V “restricted Earth return” missions;
3. Methods used to control the bioburden, decontaminate and/or sterilize the space flight hardware;
4. The organic inventory of all impacting or landed spacecraft or spacecraft-components, for quantities exceeding 1 kg;
5. Intended minimum distance from the surface of the target body for launched components, for those vehicles not intended to land on the body;
6. Approximate orbital parameters, expected or realized, for any vehicle which is intended to be placed in orbit around a solar system body;
7. For the end-of-mission, the disposition of the spacecraft and all of its major components, either in space or for landed components by position (or estimated position) on a planetary surface.

THINGS TO REMEMBER

Planetary protection is about protecting science and the Earth

- Planetary protection regulations are based on the UN Outer Space Treaty (1967);
- COSPAR maintains a planetary protection policy and associated requirements as reference for spacefaring nations;

- Planetary protection categories and requirements are not cast in stone and evolve over time as new information becomes available, i.e., check the latest version at the start of a new project.

REFERENCES

Lederberg J. and D. B. Cowie, *Science*, 1958

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, Article IX, U.N. Doc. [A/RES/2222/\(XXI\)](#) 25 Jan 1967; [TIAS No. 6347](#), 1967.

McEwen, A.S., Dundas, C.M., Mattson, S.S., Toigo, A.D., Ojha, L., Wray, J.J., Chojnacki, M., Byrne, S., Murchie, S.L., and Thomas, N., Recurrent slope lineae in equatorial regions of Mars, *Nature Geosciences*, 7, 53-58, 2014.

Rummel, J.D., Beaty, D.W., Jones, M.A., Bakermans, C., Barlow, N.G., Boston, P.J., Chevrier, V.F., Clark, B.C., de Vera, J.P., Gough, R.V., Hallsworth, J.E., Head, J.W., Hipkin, V.J., Kieft, T.L., McEwen, A.S., Mellon, M.T., Mikucki, J.A., Nicholson, W.L., Omelon, C.R., Peterson, R., Roden, E.E., Lollar, B.S., Tanaka, K.L., Viola, D., and Wray, J.J., A new Analysis of Mars “Special Regions”: Findings of the Second MEPAG Special Regions Science Analysis Group (SR-SAG2), *Astrobiology*, 14, 887-968, 2014.

Sagan C. and S. Coleman, *Astronautics & Aeronautics*, 1965

Sagan C., E. C. Levinthal, J. Lederberg, *Science*, 1968

THE COSPAR PANEL ON PLANETARY PROTECTION¹¹

COSPAR AT A GLANCE

The purpose of COSPAR, by its Charter from the International Council for Science (ICSU¹²), is to promote at an international level scientific research in space, with emphasis on the exchange of results, information and opinions, and to provide a forum, open to all scientists, for the discussion of problems that may affect scientific space research.

The COSPAR missions are mainly achieved through the organization of scientific assemblies and publications (Figure 1).

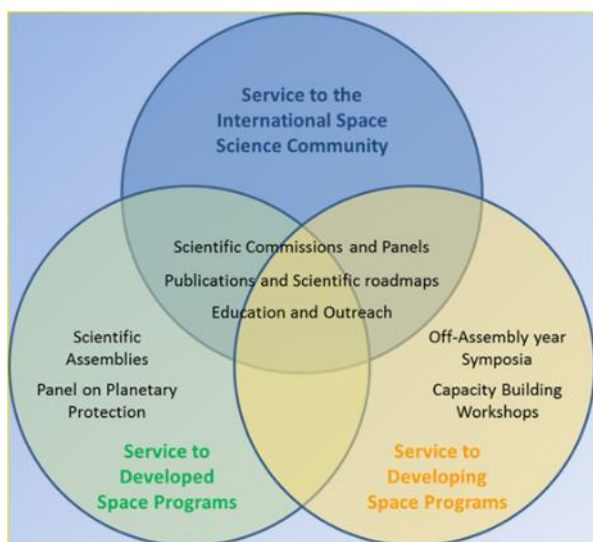


Figure 1 – The three main missions of COSPAR are represented on this graph.

The organizational structure of COSPAR consists of:

- Scientific Commissions – representing each and every scientific discipline involved in space research;
- Panels – designed to deal with crosscutting issues that can affect particular segments of the international space research community, and often for which there is an urgent need for input.

THE FRAMEWORK FOR PLANETARY PROTECTION

The legal basis and the goal for planetary protection were established in Article IX of 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 (UN Outer Space Treaty, 1967):

“...parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extra-terrestrial matter and, where necessary, shall adopt appropriate measures for this purpose...”

COSPAR maintains and promulgates a planetary protection policy (PPP) for the reference of spacefaring nations, both as an international standard on procedures to avoid organic constituent and biological contamination in space exploration, and to provide accepted guidelines and requirements in this area to guide compliance with the wording of the Outer Space Treaty. The stated aim of the policy is to fulfill the two following goals:

- **The conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized;**
- **In addition, the Earth must be protected from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from an interplanetary mission.**

THE COSPAR PANEL ON PLANETARY PROTECTION

The COSPAR Panel is concerned with biological interchange in the conduct of solar system exploration, including:

International Social Sciences Council to form the International Science Council (ISC).

¹¹ This section is based on a presentation by Dr. Gerhard Kmínek.

¹² The ICSU (formerly known as the International Council of Scientific Unions) merged in July 2018 with the

- Possible effects of contamination of planets other than the Earth, and of planetary satellites within the solar system by terrestrial organisms;
- Contamination of the Earth by materials returned from outer space carrying potential extraterrestrial organisms.

The primary objectives of the Panel within COSPAR are to develop, maintain, and promulgate planetary protection knowledge, policy, requirements and plans to prevent the harmful effects of such contamination, and through symposia, workshops, and topical meetings at COSPAR Assemblies to provide an international forum for exchange of information in this area.

At its General Assembly in 2017, the UN-COPUOS noted the long-standing role of COSPAR in maintaining the planetary protection

policy as a reference standard for spacefaring nations and guiding compliance with article IX of the Outer Space Treaty.

Through COSPAR the Panel informs the international community, e.g., the Committee on the Peaceful Uses of Outer Space (COPUOS) of the United Nations, as well as various other bilateral and multilateral organizations, of policy consensus in this area.

MAINTAINING THE COSPAR PLANETARY PROTECTION POLICY

Figure 2 provides a schematic view of the process through which the PPP (and its associated requirements) are maintained.

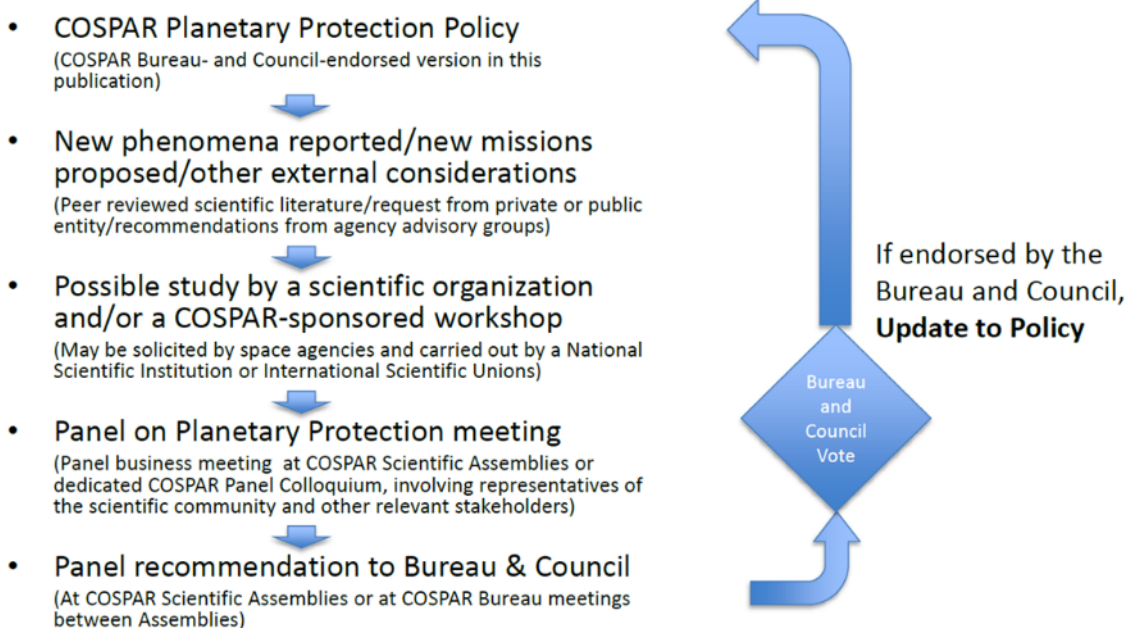


Figure 2 – A schematic representation of the process followed by the COSPAR Panel on Planetary Protection to maintain the PPP.

It is important to note that the COSPAR Planetary Protection Policy does not describe *how* to implement the requirements nor does it describe or require a certain organizational structure for the implementing entity (i.e., space agency); both aspects are under the discretion of the user (again, space agency).

Starting in July 2018, the COSPAR Panel on Planetary Protection is chaired by Dr. Athena Coustenis, with two Vice-Chairs, Dr. Niklas Hedman and Dr. Gerhard Kminek. Other members of the Panel are appointed representatives of the major space agencies involved in planetary exploration, and scientists nominated by the relevant COSPAR Scientific Commissions (SC), namely SC B on “Space

Studies of the Earth-Moon System, Planets, and Small Bodies of the Solar System” and SCF on “Life Sciences as Related to Space”.

All COSPAR events, including Panel meetings, are open fora: anyone interested can participate. But only members of the Panel can vote whenever such a procedure is required.

THINGS TO REMEMBER

- COSPAR is an international scientific committee established in 1958 by ICSU (now the ISC).
- COSPAR provides a forum for discussions and advice on matters of planetary protection since its very beginning. The CO-

SPAR Panel on Planetary Protection maintains the COSPAR Planetary Protection Policy.

- Updating the COSPAR Planetary Protection Policy is a process that includes the scientific community.
- Changes to the policy or requirements are not a one-person show, neither at COSPAR nor at the space agencies.
- The COSPAR Planetary Protection Policy is published in *Space Research Today*, COSPAR’s information bulletin (latest version in *SRT*, n°200, December 2017).

BASICS OF MICROBIOLOGY¹³

Microbiology is the study of “micro-organisms”, i.e., unicellular organisms that can be observed directly only through a microscope. Such microorganisms are very diverse and omnipresent. They can grow as single cells, cell cluster, filaments, or as biofilms.

The majority of microorganisms (Figure 3) are Prokaryotes (Bacteria and Archaea): they do not have a cell nucleus. Other microorganisms belong to the Eukaryotes with a cell nucleus, e.g., yeasts, algae, fungi, Protozoa. In addition, viruses who need a host cell for replication also belong to the group of microorganisms.

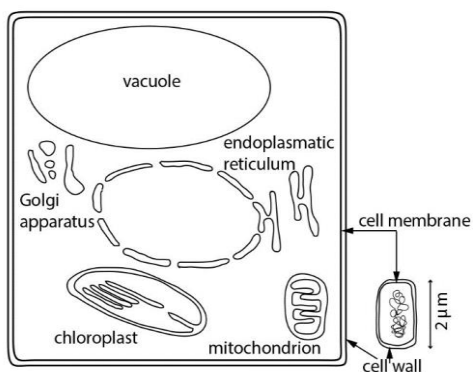


Figure 3 – (Left) Eukaryotic cell (all plants and animals). (Right) Prokaryotic cell (microorganisms).

The importance of microorganisms is illustrated by the harmful effects some of them are causing, such as:

- Propagating infectious diseases in humans, animals or plants (human, animal or plant pathogens);
- Food poisoning (production of toxic substances);
- Deterioration of materials (bio-corrosion, biofouling);
- Self-ignition of hay;
- Etc.

The importance of microorganisms is also illustrated by their equally numerous positive effects, such as:

- Decomposing organic compounds in natural nutrient cycles (decay, humus formation);
- Decomposing organic compounds in waste water (biological waste water treatment);
- Decomposing organic compounds for biogas production;
- Nitrogen fixation (for plant fertilizers);
- Acting as symbiotic bacteria in animals and plants (gut bacteria, suppression of pathogens, cellulose degradation);
- Food production (dairy products, alcoholic beverages, bread, soy sauce, ...)
- Production of antibiotics, vitamins, steroids;
- Production of biocatalysts (enzymes);
- Production of organic acids, solvents, hydrogen, ethanol, ...;
- Mining (leaching of iron, copper, uranium, ...);
- Etc.

Microorganisms changed the environmental conditions on Earth, as illustrated in Figure 4 which shows the change in the atmospheric composition as a result of biological evolution.

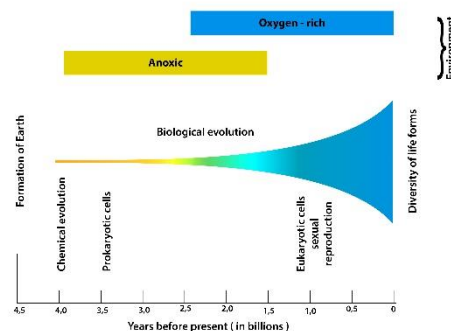


Figure 4 – Increase of the oxygen concentration in the Earth's atmosphere due to photosynthetic microorganisms.

MICROORGANISMS ARE THE OLDEST FORM OF LIFE ON EARTH

The oldest fossils found thus far on Earth are approximately 3.4 to 3.5 billion-year-old filamentous and coccoidal microbial remains in rocks of the Pilbara craton, Western Australia,

and Christine Moissl-Eichinger. Some graphics have been redrawn by Michel O. Grégoire.

¹³ This section is based on presentations from the ESA Planetary Protection course prepared by Petra Rettberg

and in rocks from the Barberton region, South Africa (Figure 5).

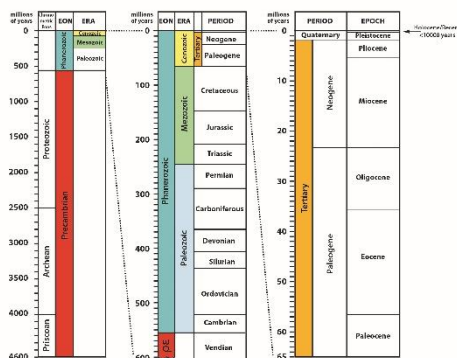


Figure 5 – Microorganisms are the oldest form of life on Earth, dated back to approx. 3.5 billions of years.

Complementing the discovery of very old microfossils, the analysis of the genetic material of all living cells can be used to trace back the emergence of life on Earth.

The sequence of the gene for 16S (or 18S, respectively) ribosomal RNA (ribonucleic acid), an important component of cell organelles necessary for protein synthesis, can be applied as a molecular clock:

This rRNA is universal, has the same function in all organisms, and changes in its sequence are presumed to occur constantly and randomly in time: the history of life can be traced back by comparing all the 16S (and 18S) sequences to the so-called ‘last universal common ancestor’ (Figure 6).

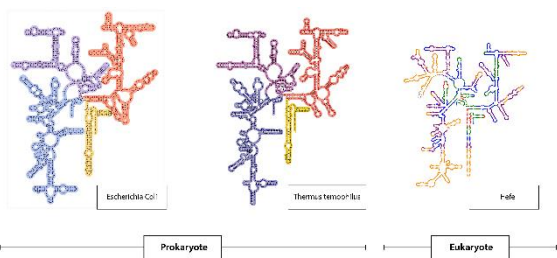


Figure 6 – The 16S RNA (in Prokaryotes) and 18S RNA (in Eukaryotes) have a common general structure and function

The ‘Tree of life’ derived from the sequence comparison shows the three domains of life (Figure 7), namely:

- Eukarya
- Bacteria
- Archaea (not discovered until the 1970s)

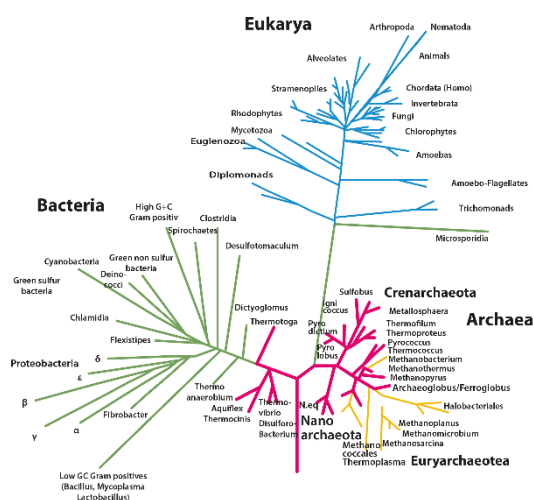


Figure 7 – The last universal common ancestor is located at the root of the phylogenetic tree (adapted from H. Huber).

SIZE, SHAPE AND STRUCTURE OF MICRO-ORGANISMS

Microorganisms that can only be observed with different types of microscopes exhibit a wide variety of sizes, shapes and structures, as exemplified in Figure 8.

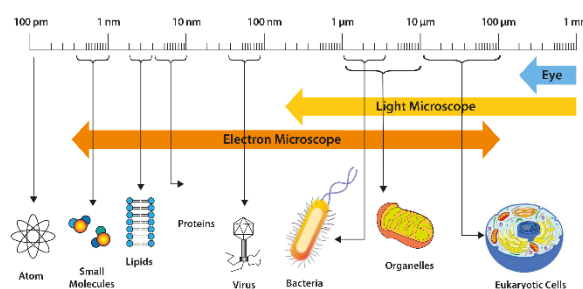


Figure 8 – Different size, shape and arrangement of bacterial cells.

Bacteria and archaea (Figure 9) are generally single-celled without nucleus. Their size ranges from ~1 to 5 μm. They have different types of cell walls. Some of them are motile.

Some bacteria have the ability to form **spores** (Figure 10), i.e., dormant, metabolically inactive, resting stages. The spore formation is triggered for example by starvation or unfavorable changes in the environment. The spores can germinate into the vegetative, metabolically-active replicating form of the organism again under more suitable conditions.

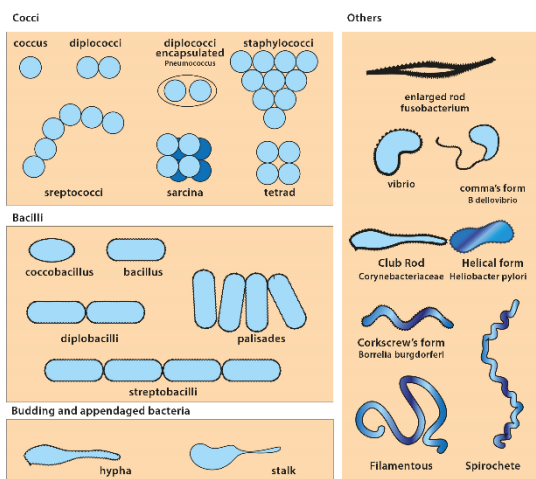


Figure 9 – Various shapes of bacteria.

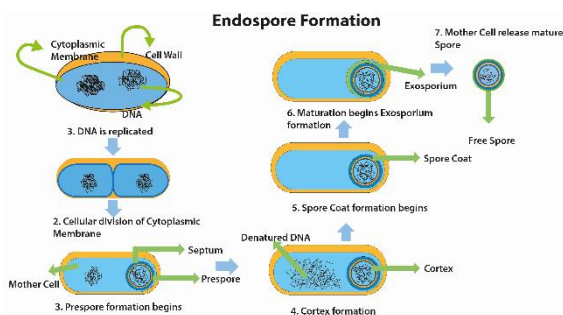


Figure 10 – The endospore formation is an active complex process to ensure the bacterial survival under unfavorable conditions.

Bacterial spores are very resistant against many physical and chemical stress factors, e.g., radiation, oxidizing compounds, desiccation, vacuum exposure, and are ubiquitous, as illustrated in Table 1.

Where	Who	Details
Sky	<i>B. stratosphericus</i>	above 24 km
Soil	<i>B. thermoterristis</i>	egypt. soil, 55°C
Hay	<i>B. subtilis</i>	the „hay“- <i>Bacillus</i>
Desert	<i>B. sonorensis</i>	Sonoran Desert, Arizona
Rocks	<i>B. simplex</i>	(500 spores/g rock)
Deep surface	<i>B. infernus</i>	
SAF*	<i>B. pumilus</i> SAFR	
Food	<i>B. cereus</i>	
Pathogens	<i>B. anthracis</i>	
Insects (pathogen)	<i>B. thuringiensis</i>	

Table 1 – Occurrence of *Bacillus* spores in extreme environments (* SAF stands for Spacecraft Assembly Facility)

A **virus** is a small infectious agent that replicates only inside living cells of other organisms. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea (then they are called bacteriophages). Virus dimensions (Figure 11 and Figure 12) lie in the range 0.02-0.3 μm. The question of whether viruses are living entities remains open.

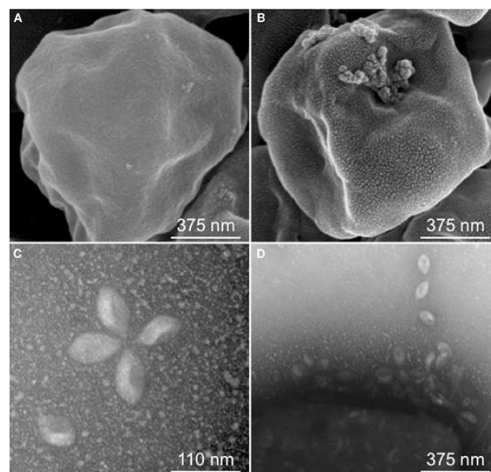


Figure 11 – *Sulfolobus* spindle-shaped virus (after Ceballos et al., 2012).



Figure 12 – Bacteriophage on a bacterial cell.

Fungi belong to the group of eukaryotic organisms with a membrane-bound nucleus (Figure 13). Fungal cells are larger than bacterial cells. Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–20 μm in diameter and up to several centimeters in length. Depending on the species fungi can reproduce sexually and/or asexually, thereby forming spores in a very complex process. These fungal spores are larger than bacterial spores and in general less resistant. One group of unicellular fungi, the yeasts, replicate by budding.

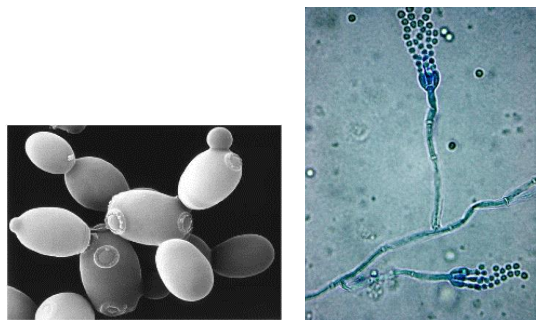


Figure 13 – (Left) Scanning electron microscopy image of *Saccharomyces cerevisiae* (Source: Rasha Aref, mpg.de). (Right) *Penicillium sp.* under bright field microscopy with lactophenol cotton blue stain.

MICROBIAL DISTRIBUTION AND ABUNDANCE

Microorganisms live almost everywhere on Earth, in ‘normal’ as well as in ‘extreme’ biotopes (Table 2). What is ‘extreme’ in this case is of course from a human perspective. ‘Extremophiles’ are organisms that thrive in physically or geochemically extreme conditions that are detrimental to most life forms on Earth. Such extremophile microorganisms can survive and live:

- At very high temperatures (hydrothermal vents, hot springs, ...);
- At very cold temperatures (permafrost, sea ice, glaciers, ...);
- At high pH values (acidic lakes, solfataras, acid mine drainage, ...);
- At low pH values (alkaline lakes, ...);
- At high salt concentrations (evaporation ponds, Dead Sea, brines in salt mines, deep sea brines, ...);

- On rocks and in the upper millimeters of porous rocks;
- In nutrient poor environments (deserts, water, cleanrooms);
- In anoxic (oxygen-free) environments (deep sea brines, deep sediments, ...);
- At low pressure (laboratory experiments);
- In high radiation environments (vicinity to nuclear reactors, laboratory experiments);
- Etc.

Where?	How many?
Garden soil, 1g	2.5×10^{10} (25,000,000,000)
Milk (raw), 1l	2.5×10^9
Air, 1 m ³	2000
Drinking water, 1 ml	< 100 (nonpathogenic)
Sea- and freshwater	1.2×10^{29}
Total (on Earth)	10^{30} (~ 10^{14} kg = 100 billion tons = 1430 billion humans (each 70 kg; currently 7 billion living humans on Earth))
Human skin	10^{12}
Human body	
- Own cells	10^{13}
- Microbes	10^{14-15}

Table 2 – Microbial distribution and abundance in various environments.

Microorganisms are also associated with human, e.g., living on the skin, or in the intestine. According to a recent estimate, 90% of cells in the human body are bacterial, fungal, or otherwise non-human. There are up to 10,000 microorganisms on 1 cm² of skin. The total number of human genes is approximately 23,000, the human microbiome encompasses more than 1,000,000 genes.

Limits	For growth	For survival
Temperature	-20°C to +113°C	≤ -263°C to +150°C
Water stress	$a_w \geq 0.7$	$0 \leq a_w \leq 1.0$ Spores survive in vacuum (10^{-6} Pa)
Salinity	Salt concentration ≤ 30 %, salt crystals	Salt crystals (endoevaporites)
pH	pH = 0-12	pH = 0 - 12.5
Nutrients	High metabolic versatility Lithoautotrophic growth High starvation tolerance	not required, better without
Oxygen	Aerobic/Anaerobic growth	not required, better without
Radiation resistance	0- high radiation resistance (< 60 Gy/h)	0- high radiation resistance (< 5 kGy)
Time	20 min up to years	≤ 25-40 × 10 ⁶ years

Table 3 – Growth and survival limits of microorganisms

Each microorganism has its specific requirements for growth. Only approximately 1% of all microorganisms can be cultivated in the lab. The parameter ranges for survival are larger than for growth, i.e., replication. The limits for growth and survival of microorganisms are illustrated in Table 3.

Microorganisms can use one or more different metabolic strategies to obtain carbon for synthesizing the cell mass, to obtain reducing equivalents used either in energy conservation or in bio-synthetic reactions, and to obtain energy for living and growing (Figure 14).

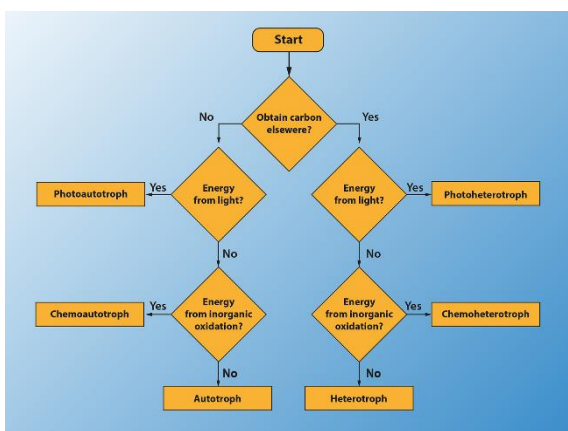


Figure 14 – Classification of microorganisms according to their metabolic characteristics

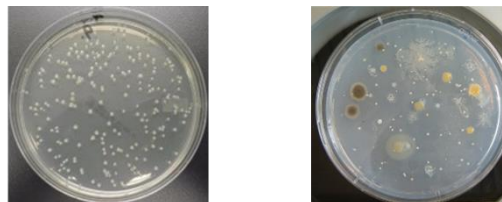
THE DETECTION OF MICROORGANISMS

Cultivation is the propagation of microorganisms with a suitable growth environment called a medium which can be solid or liquid. It may be purely chemical (a chemically defined medium) or may contain organic compounds (like yeast extract) or may consist of living organisms such as fertilized eggs. Microorganisms growing in or on such a medium form a culture.

In addition to cultivation, microorganisms can also be detected by powerful molecular methods which also identify those organisms that cannot be cultivated in the lab. High-throughput sequencing technologies and the corresponding development of bioinformatics tools for data analysis allow the fast determination

of the biodiversity in environmental samples (Table 4).

A culture is considered a pure culture if only one type of organism is present, and a mixed culture if populations of different organisms are present (Figure 15).

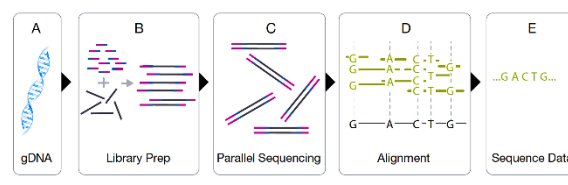


colonies from a pure culture on an agar plate

colonies from a mixed culture on an agar plate

Figure 15 – Cultivated colonies of microorganisms: pure culture (left) and mixed culture (right).

Unlike capillary sequencing or PCR-based approaches, **next-generation sequencing (NGS)** is a culture-free method that enables analysis of the entire microbial community within a sample. With the ability to combine many samples in a sequencing run, microbiology researchers can use NGS-based 16S rRNA sequencing as a cost-effective technique to identify strains that may not be found using other methods. A scheme illustrating the different steps in NGS is shown in Figure 16.



A. Extracted gDNA.
B. Sample preparation fragments genomic DNA and adds adapters to generate a library.
C. DNA fragments within the library are each sequenced in parallel.
D. Individual sequence reads are reassembled by aligning to a reference genome.
E. The whole genome sequence is derived from the consensus of aligned reads.

Figure 16 – Concept of next generation sequencing.

NGS is widely used for planetary protection, as shown in a number of scientific publications, e.g., Bashir *et al.*, 2016, Moissl-Eichinger *et al.*, 2015, Vaishampayan *et al.*, 2013, Mahnert *et al.*, 2015, La Duc *et al.*, 2009.

Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US\$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	30,000 bp (N50); maximum read length >100,000 bases ^{[63][71][84]}	87% raw-read accuracy ^[85]	500,000 per Sequel SMRT cell, 10–20 gigabases ^{[66][70][71]}	30 minutes to 20 hours ^{[66][72]}	\$0.05–\$0.08	Fast. Detects 4mC, 5mC, 6mA. ^[73]	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 600 bp ^[74]	99.6% ^[75]	up to 80 million	2 hours	\$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%	1 million	24 hours	\$10	Long read size. Fast.	Runs are expensive. Homopolymer errors.
Sequencing by synthesis (Illumina)	MiniSeq, NextSeq: 75–300 bp; MiSeq: 50–600 bp; HiSeq 2500: 50–500 bp; HiSeq 3/4000: 50–300 bp; HiSeq X: 300 bp	99.9% (Phred30)	MiniSeq/MiSeq: 1–25 Million; NextSeq: 130–00 Million, HiSeq 2500: 300 million - 2 billion, HiSeq 3/4000 2.5 billion, HiSeq X: 3 billion	1 to 11 days, depending upon sequencer and specified read length ^[76]	\$0.05 to \$0.15	Potential for high sequence yield, depending upon sequencer model and desired application.	Equipment can be very expensive. Requires high concentrations of DNA.
Sequencing by ligation (SOLiD sequencing)	50+35 or 50+50 bp	99.9%	1.2 to 1.4 billion	1 to 2 weeks	\$0.13	Low cost per base.	Slower than other methods. Has issues sequencing palindromic sequences. ^[77]
Nanopore Sequencing	Dependent on library prep, not the device, so user chooses read length. (up to 500 kb reported)	~92–97% single read	dependent on read length selected by user	data streamed in real time. Choose 1 min to 48 hrs	\$500–999 per Flow Cell, base cost dependent on expt	Longest individual reads. Accessible user community. Portable (Palm sized).	Lower throughput than other machines, Single read accuracy in 90s.
Chain termination (Sanger sequencing)	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	\$2400	Useful for many applications.	More expensive and impractical for larger sequencing projects. This method also requires the time consuming step of plasmid cloning or PCR.

Table 4 – Comparison of high-throughput sequencing methods (https://en.wikipedia.org/wiki/DNA_sequencing)

THINGS TO REMEMBER

- **Microorganisms** are (almost) everywhere.
- They can survive extremely harsh conditions.
- Some microorganisms can form spores.
- Bacterial spores are resistant to many physical and chemical stressors.
- Each microorganism has specific requirements... only about 1% of all microorganisms are cultivable in a laboratory.
- Different cultivation-independent high-throughput methods can be used to study the microbial diversity.
- Most microbial contaminants in **spacecraft assembly cleanrooms** are human-associated.
- Cleanroom isolates can be more resistant than comparable laboratory strains of the same species.

- In cleanrooms, spore-formers are present as spores.
- A broad diversity of microorganisms is present in cleanrooms, with different adaptations.
- The microbial contamination is not homogeneously distributed in a cleanroom.

More information about microorganisms can be found in general microbiology textbooks, e.g. Madigan *et al.*, 2018.

REFERENCES

Bashir M., *et al.*, 2016, doi:10.3389/fmicb.2016.01321.

Ceballos, R., Marceau, C., Marceau, J., Morris, S., Clore, A.J., and Stedman, K. (2012). Differential Virus Host-Ranges of the *Fuselloviridae* of *Hyperthermophilic Archaea*: Implications for Evolution in Extreme Environments.

Frontiers in microbiology. 3:295, 10.3389/fmicb.2012.00295.

La Duc, M.T., *et al.*, 2009, doi: 10.1128/AEM.01073-09.

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., and Stahl, D. A. (2018), Brock Biology of Microorganisms. Ed. by Pearson; 15th edition (28 March 2018), ISBN-10: 1292235101, ISBN-13: 978-1292235103

Mahnert, A., *et al.*, 2015, doi: 10.1371/journal.pone.0134848.

Moissl-Eichinger, C., *et al.*, 2015, doi: 10.1038/srep09156

Vaishampayan, P.A., *et al.*, 2013, doi: 10.1038/ismej.2012.114.

ORGANIC CONTAMINATION CONTROL¹⁴

ORGANIC CONTAMINATION

Organic molecules are carbon-containing compounds which are the building blocks in biological systems. As such, organic molecules are often considered proxies for the presence of life, biomarkers. Therefore, the search for extra-terrestrial life concerns itself with their detection. Yet biological activity is not the only source of organic compounds, and abiotic or prebiotic chemistry can also produce organic molecules. Thus the search for organic molecules is crucial for our understanding of the chemistry of the cosmos, as well as our place within it.

In attempts to analyze extra-terrestrial molecules we want to detect organics indigenous to the body of interest rather than just terrestrial contamination introduced through our process of exploration. This is where organic contamination control – the process of restricting the delivery of terrestrial organics to the target body, via the spacecraft, comes in.

Organic contaminants are any organic molecules which are on/in the spaceflight hardware and which may be detected by the analytical technique(s) used. They may be either particulate and/or molecular and have been introduced to the system in numerous ways including: atmospheric fallout or surface transfer during the build/assembly stages, impurities in materials used in the build or been created by breakdown of materials during the flight as they are exposed to vacuum, extreme temperature and radiation environments.

Biological contamination, is a subset of organic contamination as we detect organic molecules derived from microbial cells (whether they are alive or dead). However, not all organic contamination is biological (Figure 17) as organic molecules may come from numerous non-biological sources.

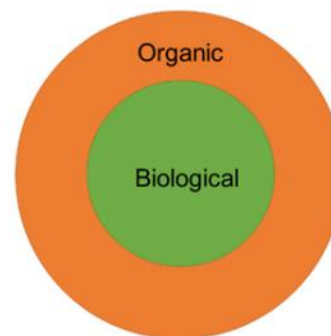


Figure 17 – All biological contamination (microbial) is organic but not all organic contamination is biological.

47

IMPORTANCE OF ORGANIC CONTAMINATION CONTROL

For the current mission, the organic contamination control concern for planetary protection is that contamination will lead to incorrect conclusions being drawn from data collected during the mission: either a false positive or a false negative. A false positive is when contamination is mistaken for the true presence of a compound of interest (e.g., a proxy for life, a biomarker, in a life detection mission). A false negative is when a high instrument background, due to terrestrial contaminants, masks a low signal from a compound of interest that is actually present in the sample – making it impossible to detect. This is illustrated in Figure 18.

Analysis result	The Truth	
	Life	No life
Positive (detection)	True Positive	False Positive
Negative (no-detection)	False Negative	True Negative

The diagram also includes a yellow oval labeled 'Bad' covering the True Positive and False Positive cells, and a red oval labeled 'Good' covering the True Negative cell.

Figure 18 – How false positives and false negatives relate to analysis results and the true nature of the analyzed samples. An instrument needs to be precise with a minimal number of false results – illustrated by the narrower 'Good' ellipse.

The questions of how much contamination is acceptable and what contaminants are problematic are highly specific to both the analytical instrument(s) and the target body. Contaminants are not a problem for a mission if they

¹⁴ This section has been contributed by Samuel H Royle and Mark A. Sephton, Imperial College London.

either cannot be measured by the analytical technique(s) nor if they are easily identifiable as contaminants. The necessary cleanliness of the instrument is also governed by the expected levels of the compounds of interest on the target body, if the expected levels of organic compounds will be low the instrument must be cleaner to reduce the signal to background noise ratio but if there will be plenty then the background can be higher and still obtain a positive detection (Figure 19).

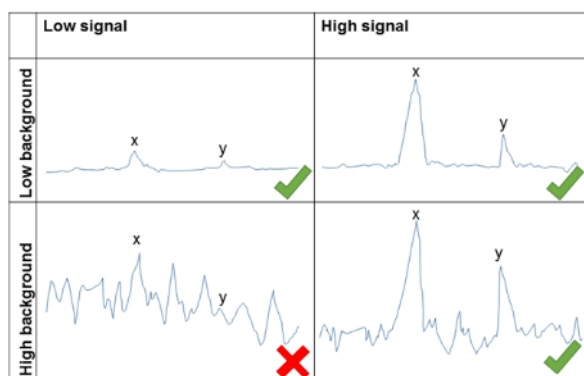


Figure 19 – Signal to background noise ratios, to detect compounds x and y they must be present at significantly higher quantities than the background. To reduce the background, and therefore increase the sensitivity of the instrument, we must have less contamination.

Unlike with biological contamination, forward contamination is less of an issue for organic contamination control as the organic molecules will not self-replicate (which is the potential worry with microbial contamination) and instead are more likely to be diluted to very minute concentrations if they are spread across the body of interest and so will not be detectable by future missions. However, as it is expected that scientific instruments sent out in the future will be more sensitive and therefore have lower detection thresholds than the current limits, this is still something we need to consider.

PRACTICES OF ORGANIC CONTAMINATION CONTROL

Organic contaminants may come from the materials used in the build phase. Plastics, resins, polymers, oils and lubricants may all contain and release organic compounds which, if not properly shielded, may contaminate sensitive

areas of the spacecraft. Unless sufficient barriers can be put in place to protect the instrumentation or sample from contamination, materials need to be selected that will not induce contamination that exceeds the allowed levels. Solid materials may contain impurities which are released to the environment by outgassing during exposure to vacuum, especially at elevated temperatures. Materials must be non-flaky, non-dusty, shatterproof and be resistant to outgassing under the expected conditions of the mission (e.g., temperature and radiation environments). Materials selected will also need to be able to withstand any contamination control cleaning procedures (e.g., high temperatures during thermal bake out).

Effective cleaning protocols need to be in place. These will be a series of suitable cleaning steps throughout the instrument and spacecraft build and assembly phases to reach and maintain required cleanliness levels. Repeated wiping down with solvents of diverse polarity, ultrasonic cleaning, mechanical cleaning, thermal bake out under vacuum, plasma cleaning, accelerated CO₂ snow are all accepted techniques (e.g., Task Group on the Forward Contamination of Europa, 2000; Committee on preventing the forward contamination of Mars, 2006; ten Kate *et al.*, 2008; Dworkin *et al.*, 2018), the selection of which will depend on the contaminants of interest and the compatibility of the materials being cleaned. Procedures also need to be in place to monitor the effectiveness of these techniques and for mitigation in the case of accidental recontamination.

Maintaining relevant clean room practices with the use of air filters, positive air pressure, protective clothing and barriers/covers to isolate the most sensitive parts of the instruments allows cleanliness levels to be kept high and prevent recontamination until launch. Monitoring to test for potential recontamination with the use of witness plates to check atmospheric fallout and surface swabbing is necessary.

As it is currently not possible to build a spacecraft that is completely free from materials that may introduce organic contamination, contam-

ination knowledge is as important as contamination control. Keeping archives of all materials used in the build to experiment on makes it quicker and easier to identify contaminants in the produced data by replicating analyses on the ground (Dworkin *et al.*, 2018). Modelling how contaminants will be produced (e.g., by degassing and exposure to extreme radiation and thermal environments) and transported throughout the life of the mission is particularly important and challenging (ten Kate *et al.*, 2008).

HISTORY OF ORGANIC CONTAMINATION CONTROL

The introduction of contamination control in the space exploration program originated not from concerns over reliable data but from a more fundamental reliability issue with the worry that missions could fail because particulate matter (i.e., dust) could interfere with the operation of highly precise electronic, hydraulic, electromechanical or electro-optical systems. For example, causing a valve to stick in a propulsion system, resulting in that control of the spacecraft being lost. This concern led to the implementation of the first NASA clean-room technology in the 1950s (Useller, 1969).

The earliest data-related contamination control worries were in the late 1950's in the wake of Sputnik and subsequently in the run up to the Apollo lunar missions. It was realized that forward terrestrial contamination from spacecraft could compromise future scientific experiments and so the Committee on Contamination by Extraterrestrial Exploration (CETEX) was established (Meltzer, 2011). With little known about the Moon at the time, it was seen as important that terrestrial contamination was kept as localized as possible during the lunar landings in case interesting prebiotic chemistry was irreversibly damaged. With further missions to the Moon leading to the discovery that the Moon is relatively uninteresting from a biology/organic geochemistry point of view, contamination control became a much lower priority for subsequent missions.

At the time of planning the Viking missions, Mars was thought to be much more similar to

Earth, and therefore potentially habitable and so contamination control for the Viking missions was taken very seriously. The main contamination focus was on biological contamination due to the concern of terrestrial microbial life being released into, and proliferating on, the Martian surface, with unpredictable consequences following interaction with indigenous Martian life. However, organics were still taken into consideration, with potential sources of contaminants analyzed; maximum allowable amounts of terrestrial contamination based on the sensitivity of the instruments set; and approaches to minimizing contamination throughout the design, build and operation stages planned at the early stages of mission development (Flory *et al.*, 1974). Viking level cleanliness is still used as the benchmark for life-detection missions to Mars.

The most recent mission to the Martian surface, the Mars Science Laboratory (MSL) on the Curiosity Rover was equipped with the Sample Analysis at Mars (SAM) instrument suite, able to detect organic molecules with sub-parts-per-billion sensitivity. While not strictly a life-detection mission, this high sensitivity meant that high organic cleanliness of the instruments and sample handling chain were paramount. To direct this effort, NASA commissioned an Organic Contamination Science Steering Group (OCSSG) which developed strategies for both the engineering and operations teams to reduce organic contamination down to acceptable levels (ten Kate *et al.*, 2008) and for the science teams responsible for data analysis so that they could recognize any contaminants detected in the data (Mahaffy *et al.*, 2003).

CURRENT CHALLENGES IN ORGANIC CONTAMINATION CONTROL

Organic contamination control concerns will differ greatly depending on the environment of the target body. The exploration of the Icy Moons of the Outer Solar System is still very much in its infancy and there are many unknowns in the factors to be considered.

All of our experience in organic contamination control in the context of planetary protection so

far have been tailored to the Martian Environment, which, in comparison to the Icy Moons, is now relatively well understood.

In particular, the radiation environment on the surface of the Icy Moons is yet to be constrained and it may well be that it will cause the alteration of terrestrial contaminants into unexpected forms. As this radiation environment is expected to also have created a complex suite of polymers and macromolecules from the plentiful organics supposed to be present at the Icy Moon surface (Johnson *et al.*, 2012; Kimura and Kitadai, 2015), it will be hard to distinguish these modified terrestrial contaminants, indigenous/exogenous non-biological products of this environment, and any indigenous biomarkers which may be present from each other. The presence, on the Icy Moons, of abundant liquid water (Waite Jr *et al.*, 2009) and its unknown solute chemistry may also have significant effects.

There are still substantial general knowledge gaps presenting challenges in organic contamination control implementation. These include a lack of a rapid universal method for verification of cleanliness, acceptable contamination levels for specific instruments and statistical methods for analytical certainty. Constant adaptation and modification of existing contamination control protocols will be necessary for incremental improvements in this field as knowledge processes.

REFERENCES

- Committee on preventing the forward contamination of Mars (2006): *Preventing the Forward Contamination of Mars*, National Academies Press, Washington, D.C.
- Dworkin, J.P. *et al.* (2018): OSIRIS-REx Contamination Control Strategy and Implementation. *Space Science Reviews*, 214, DOI: 10.1007/s11214-017-0439-4.
- Flory, D.A., J. Oro and P.V. Fennessey (1974): Organic contamination problems in the Viking molecular analysis experiment. *Orig. Life*, 5(3–4), 443–455, doi:10.1007/BF01207643.
- Johnson, P.V., R. Hodyss, V.F. Chernow, D.M. Lipscomb and J.D. Goguen (2012): Ultraviolet photolysis of amino acids on the surface of icy Solar System bodies. *Icarus*, 221(2), 800–805, doi:10.1016/j.icarus.2012.09.005.
- ten Kate, I.L., J.S. Canham, P.G. Conrad, T. Errigo, I. Katz and P.R. Mahaffy (2008): Mitigation of the Impact of Terrestrial Contamination on Organic Measurements from the Mars Science Laboratory. *Astrobiology*, 8(3), 571–582, doi:10.1089/ast.2007.0160.
- Kimura, J., and N. Kitadai (2015): Polymerization of Building Blocks of Life on Europa and Other Icy Moons. *Astrobiology*, 15(6), 430–441, doi:10.1089/ast.2015.1306.
- Mahaffy, P. *et al.* (2003): *Report of the Organic Contamination Science Steering Group*.
- Meltzer, M. (2011): *When biospheres collide : a history of NASA's planetary protection programs*, NASA, Washington D.C.
- Task Group on the Forward Contamination of Europa (2000): *Preventing the Forward Contamination of Europa*, National Academies Press, Washington, D.C.
- Useller, J.W. (1969): *Clean room technology*, Washington, D.C.
- Waite Jr, J.H. *et al.* (2009): Liquid water on Enceladus from observations of ammonia and 40Ar in the plume, *Nature*, 460(7254), 487–490, doi:10.1038/nature08153.

BIOBURDEN, ASSAY AND STERILIZATION¹⁵

MICROBIAL DIVERSITY

Applying the biodiversity considerations highlighted in the Basics of Microbiology section to the spacecraft environment, it is found that spacecraft are exposed to many organisms during their pre-flight assembly. Figure 20 provides an example of the microbial diversity observed in clean rooms (percentages are listed in Table 5).

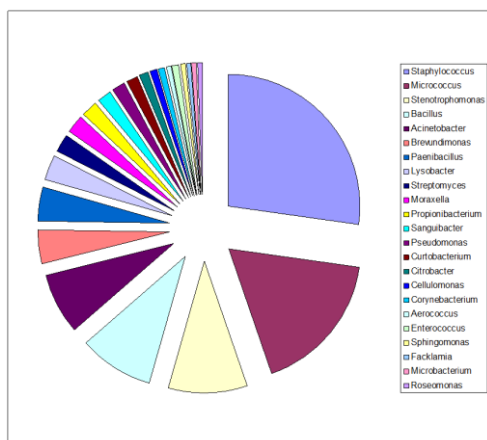


Figure 20 – Example for cultivable diversity at genera level in cleanrooms (Courtesy C. Moissl).

Microbial diversity includes differential resistance to sterilization processes. Correspondingly lethality (accounted by D10 value – see below) of dry heat for spore formers may vary from a few minutes to several tens of hours depending on the species. The kinetics of this lethality (for both individual species and for communities of bacteria) typically follow an Arrhenius relationship (Figure 21).

The hierarchy of resistance for dry heat processes (from Low to High) for microorganisms typically follows this series:

- Viruses (Influenza, HIV)
- Bacteria, fungi (Salmonella, yeast)
- Fungal spores (Molds)
- Non-enveloped viruses (Hepatitis A)
- Bacterial spores (Anthrax, tetanus)
- Prions (BSE).

This is the general case. However, exceptions and variability are the rule in biology. In the case of spacecraft bioburden management, it is usually the (relatively resistant) spore forming bacteria that are the indicator organisms (although for Icy Moons, other organisms might also be important, for example radiation resistant organisms sent to the Jovian system that might end up at Europa).

Genus	Percentage of total isolates
Staphylococcus	25.6
Micrococcus	16.5
Stenotrophomonas	9.0
Bacillus	8.7
Acinetobacter	6.9
Brevundimonas	4.0
Paenibacillus	3.8
Lysobacter	2.9
Streptomyces	2.2
Moraxella	2.0
Propionibacterium	1.8
Sanguibacter	1.8
Pseudomonas	1.4
Curtobacterium	1.3
Citrobacter	1.1
Cellulomonas	0.9
Corynebacterium	0.7
Aerococcus	0.7
Enterococcus	0.7
Sphingomonas	0.5
Facklamia	0.5
Microbacterium	0.5
Roseomonas	0.5

Table 5 – Percentages of total isolates shown in Figure 20.

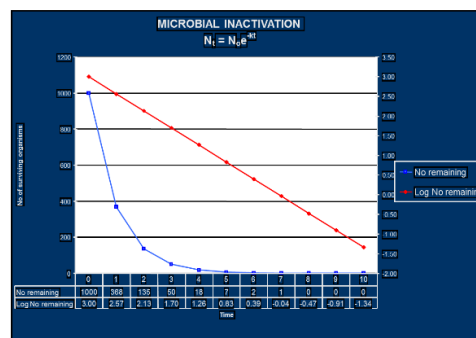


Figure 21 – Kinetics of sterilization: number of surviving organisms as a function of time.

¹⁵ Section based on a presentation by Dr. J Andy Spry.

STERILIZATION TERMINOLOGY

In considering sterilization, it is appropriate to address specific the definitions of terms, which have more precise meaning in this application than in general speech or other industries:

- **Sterile:** Free of living organisms, including bacterial spores, and may include infectious substances (e.g., virus, prions) inactivation.
- **Sterilization:** A process (rather than an end point) that destroys or eliminates living organisms, including bacterial spores, and infectious substances.
- **Disinfection:** Surface process that destroys vegetative (i.e., able to multiply) forms of harmful microorganisms, generally excluding spores.
- **Pasteurization:** Heat process that arrests microbial growth but not necessarily lethal to all cell types.
- **Inactivation:** Loss of ability of microorganisms to grow and/or multiply.
- **D-value or D10:** Time or dose required for a process to achieve inactivation of 90% of a test population (i.e., 1-log reduction).
- **z-value:** Number of degrees required to change the D-value by 1-log.
- **(D)HMR:** (Dry) Heat Microbial Reduction, the typically preferred method for bioburden control of spacecraft hardware.
- **VHP:** Vapor Hydrogen Peroxide an alternative method for bioburden control of spacecraft hardware.

BIOBURDEN TERMINOLOGY

Similarly, in considering management of spacecraft microbial cleanliness, it is appropriate to address the definitions of terms typically used in the planetary protection endeavor:

- **Bioburden:** Quantity of viable microorganisms on a product detected with a specific assay.
- **Biodiversity:** Identification of the spectrum of microorganisms on an item detected using a specific assay.

- **Biological indicator:** Test system containing viable microorganisms providing a defined resistance to a specified sterilization process.

Surface bioburden:

- **Bioburden on exposed surfaces,** i.e., bioburden that can either redistribute to other parts of the S/C or bioburden that can be released to the environment
- **Mated bioburden:** Bioburden between a matched join by fasteners rather than by adhesives.
- **Encapsulated bioburden:** Bioburden buried inside non-metallic materials, i.e., not free for gas-exchange.

STERILIZATION STANDARDS

The medical industry has developed a set of standards and approaches that are relevant to developing a spacecraft sterilization process, and can be a resource for spacecraft managers and engineers, alongside ECSS and other documents described below:

- ISO 11138: Sterilization of health care products – biological indicators
- ISO 11137: Sterilization of health care products – radiation
- ISO 17665: Sterilization of health care products – moist heat
- ISO 11607: Packaging of terminally sterilized medical devices
- ISO 20857: Sterilization of health care products – dry heat

BIOBURDEN ON SPACE CRAFT**THE BIOBURDEN REDUCTION PROCESS OPTIONS**

In preparing a planetary protection implementation strategy to reduce the bioburden, one needs to consider:

- Chemical vs. physical processes.
- Surface vs. bulk processes.
- Release criteria (parametric or verification of efficacy).
- Short and long-term (materials) effects.
- Recontamination prevention (packaging, storage, inventory).

The decision on which option to choose will be dependent on the individual spacecraft and its mission.

Type	Methods	Sterilization type		Heritage	
		Surface	Bulk	Studied	Studied and used
CHEMICAL	Formaldehyde gas	X	--	Space components (US 1965)	--
	Ethylene oxide (EtO)	X	--	--	Ranger 1961/62
	Sporicidal solution (TED)	X	--	Mars 96	Mariner Mars 1971
	Hydrogen peroxide	X	--	--	Mars96, Beagle2, DS2
THERMAL	Dry Heat	X	X	--	Viking, Mars96, Pathfinder, Beagle2, MEP, Phoenix, MSL
STEAM	Steam (space hardware excluded)	X	--	--	Excluded on space h/w, only GSE, garments
RADIATIVE	Gamma / Beta radiations	X	X	--	Mars96, Beagle2

Table 6 – Bioburden reduction type and mission examples

Table 6 illustrates sterilization process choices used by various interplanetary missions. Note that a particular project could use more than one process at different times in the project timeline and/or for different hardware elements of the spacecraft...

In selecting a Bioburden Reduction Process, one also needs to consider:

- Product (single/multi component, level of assembly, geometry);
- Material compatibility (consult local Subject Matter Experts):
 - Significant issues around variability;
 - Always preferable to test the flight batch/article.
- Biological efficacy (validated log-reduction, pre/post-process conditioning).

BIOBURDEN REDUCTION PROCESS SPECIFICATIONS

Standard process specifications are available for (D)HMR and VHP, that can be used parametrically, without further need to optimize or qualify. Briefly, these are, respectively:

- 110-200°C, +/- humidity control < 1.2 g/m3, D1₁₀ (hrs) for 2-3 log reduction, D2₁₀ (hrs) for 4-6 log [hardy] reduction for heat processing – see ECSS-Q-ST-70-57C).
- 25-45°C, vacuum at 1-10 torr or controlled humidity, 3-50%, concentration of 0.5-1.1 mg/L, D10 = 200 (mg/L).sec, acceptable range 2-6 log reductions for VHP processing – see ECSS-Q-ST-70-56C).

Note that:

- Deviation from specification requires approval.
- Use of other bioburden reduction processes (e.g., ionizing radiation) can be negotiated and is subject to approval.
- To protect budget and schedule, using the synergy of bioburden reduction and, e.g., contamination control bake-out processes is recommended.

The standard processes are described in more detail in the following section

STERILIZATION TECHNOLOGIES AND BIOBURDEN REDUCTION

The heat reduction processes have recently been modified, based on updated lethality information (Schubert *et al.*, 2012). Missions from Viking up until MSL used the “old” method. NASA missions from InSight onward have used the new specifications.

DRY HEAT MICROBIAL REDUCTION (DHMR) PROCESS – OLD STYLE

This process is implemented in a narrow temperature range based on conditions used for Viking and microbiological data generated in that era. The temperature is constrained to between 104-125°C, with additional credit to 146°C negotiable with the PP Officer.

DHMR is based on logarithmic reduction in microbial survival with increasing amount of heat process:

$$\log N_{(DHMR \text{ at time } t)} = \log N_{(preDHMR)} - t/D$$

where D is the time to reduce the microbial population by 90%, given as $(5 \times 10^{((125-T)/21)})$ for the reference organism (encapsulated), t = time (hrs.), and T = Temp (C).

The DHMR process requires stringent control of humidity. It is capped at 4 logs of reduction, based on the occurrence of so-called “hardy” organisms. Viking landers accounted ~300,000 spores prior to terminal DHMR and ~30 spores after DHMR. This is the source of the 300,000 specification number for landed spacecraft today as per Barengoltz, 1989.

The NASA Mars Program supported generation of experimental data in the 2005-2011 timeframe to support expansion of:

- Permitted temperature range.
- Permitted humidity control environments.
- Maximum permitted log reduction credit.

Experimental data from USA and Europe support the revision of all three parameters. Revision is also needed to take into account increased understanding of the terrestrial biosphere.

NEW (D)HMR PROCESS SPECIFICATIONS

The good news for (D)HMR:

- Bioburden reduction credit is allowed for processes up to 200°C (including manufacturing environments).
- It is permitted to use ambient humidity processes (simplifies the requirement and reduces the cost for HMR by allowing use of ambient humidity ovens instead of vacuum ovens).
- There is opportunity to reduce mission costs associated with not having to reach 500°C for 0.5 seconds before bioburden reduction credit can be obtained for atmospheric entry heating in break up and burn up analyses.
- Increased bioburden reduction credit beyond the four order of magnitude reduction limit can be accounted.
- The new process facilitates spacecraft hardware manufacturing being able to achieve sterility (accounting < 1 [“zero”] survivor organisms).

Table 7 shows a comparison between the old DHMR approach and the current practice.

Old Approach DHMR (per NPR8020.12D)	New Approach (D)HMR (Current NASA Practice)
<ul style="list-style-type: none"> • Surface Minimum Temp.: <ul style="list-style-type: none"> - 1-4 log reduction: 104°C - 5-6 log reduction: N/A 	<ul style="list-style-type: none"> • Surface Minimum Temp.: <ul style="list-style-type: none"> - 2-3 log reduction: 110°C - 4 log reduction: 110°C - 5-6 log reduction: >125°C
<ul style="list-style-type: none"> • Encapsulated Minimum Temp.: <ul style="list-style-type: none"> - 1-4 log reduction: 111°C - 5-6 log reduction: N/A 	<ul style="list-style-type: none"> • Encapsulated Minimum Temp.: <ul style="list-style-type: none"> - 2-3 log reduction: 110°C - 4 log reduction: 110°C - 5-6 log reduction: >125°C
<ul style="list-style-type: none"> • Surface & Encapsulated Maximum Temp.: 125°C (146°C by exception) 	<ul style="list-style-type: none"> • Surface & Encapsulated Maximum Temp.: 200°C

Table 7 – Comparison of HMR Implementation

There are also some bad news aspects of the new process:

- For a standard “4 log” reduction, the process time will be substantially longer at the same temperature, OR, the temperature of process will need to be hotter for the same time duration.
- For Phoenix/InSight hardware for example: for surface Bioburden (mated surfaces): in general, the minimum PHX bake-outs were 112°C, 37 hrs; for InSight, a comparable minimum. bake-out would be 112°C, 132.2 hrs.
- With choice comes complexity: (D)HMR becomes an implementation and management challenge.

THE VAPOR HYDROGEN PEROXIDE (VHP) PROCESS

In this process (represented graphically in Figure 22), VHP Generator systems initially dehumidify the ambient air in the sterilization chamber, then produce VHP by passing aqueous hydrogen peroxide over a vaporizer, and circulate the vapor at a programmed concentration in the air, (typically from 140 ppm to 1400 ppm).

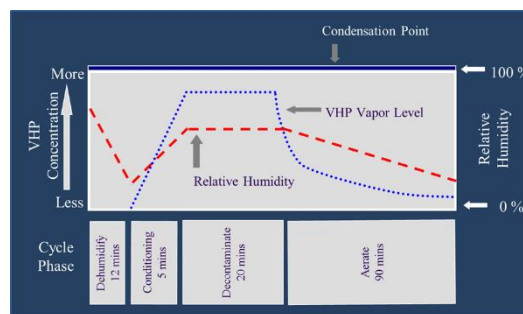


Figure 22 – The VHP Process timeline.

By comparison, a concentration of 75 ppm is considered by NIOSH to be “Immediately Dangerous to Life or Health” in humans.

The VHP maximum vapor level and corresponding decontamination time has been validated by JPL using a 1 mg/L, 30 min process (1800(mg/L).sec = 9 log reduction).

The VHP Process must still be matured for flight applications. Process electronic and mechanical parts need to be validated for aseptic

assembly applications: micro-D flight connectors without pigtailed; bolted anodized and untreated aluminum plates are examples of complex geometries that can be used to validate VHP performance. As developed, the process can be deployed in a variety of configurations, based on the hardware to be sterilized, from room size sterilization applications through benchtop cabinets to individual bagged parts such as printed wire boards.

ACCOUNTING AND ASSAY

APPLICATION OF A BIOBURDEN REDUCTION PROCESS

Applying a Bioburden Reduction Process implies a Quality System. To ensure efficacy in implementation and accounting of bioburden (reduction), one needs to consider:

- Equipment Certification
- Maintenance
- Calibration of sensors
- Training
- Accessibility
- Documentation/record keeping
- Audits

COMPATIBILITY

As well as efficacy, compatibility of the hardware with the process also needs to be demonstrated. For part/component compatibility tests, one needs to know the parameters that need to be tested (strength, surface reflectance etc.). In particular, one needs to pay attention in the implementation phase to deltas between as-designed (when the sterilization approach will have been determined) and as-built.

Further, one needs to perform integrated compatibility tests (incl. parameters such as CTE), and process cycle definition on development models. Validation of process and qualification of product on qualification model is also needed. The process cycle must be applied to the flight model (and spare).

PRECONDITIONING

Dirty products cannot be sterilized effectively and reliably. One should achieve a pre-sterilization cleanliness level of visibly clean (which

should be formally specified). IPA or ethanol cleaning can be used (may not be sporicidal). One must pay attention to grade (residue) and SMAC. ESA has shown that additives (e.g., a few % H₂O₂) can improve biological efficacy of cleaning, reducing the initial pre-sterilization bioload – contact time is important if this additional credit is taken and a standardized process needs to be developed and adopted.

BIOBURDEN ASSESSMENT

For process selection and planning, bioburden specification per cleanroom class can be used (table in NPR8020.12 and ECSS documents).

For application of process on flight H/W, a pre-process assay is usual.

Assay procedures are available in NASA HDBK6022 or in ECSS documents.

PREVENTING RECONTAMINATION

Isolation of a cleaned item (e.g., spacecraft) from a less clean environment (e.g., launch vehicle fairing) by an enclosure or “biobarrier” is necessary to preserve cleanliness levels.

Such isolation approaches are also important for cleanliness preservation during e.g., storage prior to integration, transportation between cleanrooms and/or test facilities.

An end-to-end recontamination approach may use flight (deployable) biobarriers for prevention of recontamination and/or non-flight (temporary/ disposable) biobarrier items during assembly.

THINGS TO REMEMBER

Bioburden, Assay and Sterilization Key Points include:

- Determine the bioburden allocations;
- Know the manufacturing processes and environments;
- Select the most appropriate stage(s) in the assembly sequence for applying bioburden reduction;
- Know the bioburden and biodiversity on the product;
- Select a process, paying attention to material compatibilities;

- Integrate the process in the product development and test plan;
- Clean before you apply a bioburden reduction process;
- Pay attention to appropriate recontamination prevention;
- Don't forget the spares;
- If things go wrong, e.g., re-work, make sure that product can either take more cycles (part of qualification program) or use spares.

REFERENCES

Barengoltz, J., Particle Adhesion to Surface Under Vacuum. *Journal of Spacecraft and Rocket*, 26(2), p. 103. 1989.

Schubert, W.W., Spry, J.A., Ronney, P.D., Pandian, N.R. and Welder, E., Experimental Modeling of Sterilization Effects for Atmospheric Entry Heating on Microorganisms, NASA Tech Brief, NPO-48091, 2012.

ORBITAL DYNAMICS AND IMPACT PROBABILITY ANALYSIS¹⁶

OVERVIEW

This presentation mainly focuses on the following issues regarding planetary protection: How to prove that a mission satisfies the requirement from planetary protection policy quantitatively? And what should we care about in the mission design?

These issues are discussed in the context of the Hayabusa-2 mission, the 2nd Japanese sample return mission to a small body, but the analysis method and process can be applied to other missions in general.

Hayabusa-2 was launched by JAXA in December 2014, reached its target, the C-type asteroid Ryugu, in June 2018 and will return back to the Earth in 2020. It is an interplanetary spacecraft, and the planetary protection policy had to be taken into account in such a mission.

Hayabusa-2 Mission Schedule

Earth Departure: December, 2014
 Earth Swing-by: December, 2015
 Ryugu Arrival: June, 2018
 Ryugu Departure: December, 2019
 Earth Reentry: December, 2020

HAYABUSA-2 AND PLANETARY PROTECTION

When designing an interplanetary mission, one must consider and obey planetary protection policy, in order not to contaminate planets where the origin of life may exist (forward contamination), and not to endanger the Earth by bringing extraterrestrial organisms, if such exist (backward contamination). In this study, the forward contamination is more important to be considered.

COSPAR has developed recommendations aimed at avoiding interplanetary contamination. In particular, Mars, Europa, Enceladus

are targets that should be taken care of in consideration of potential life on these bodies.

According to the destination and spacecraft type (e.g., orbiter, flyby, sample return...), the mission is categorized into one (or more than one) “Category” defined in the COSPAR Planetary Protection Policy.

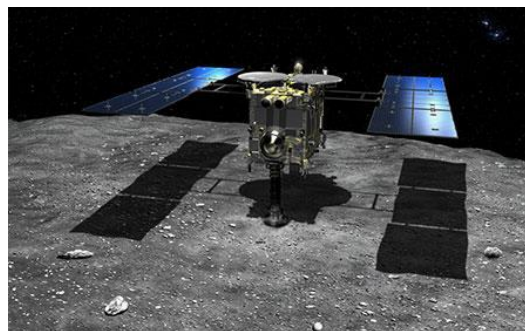


Figure 23 – Hayabusa-2 artist rendition
 (Credit: JAXA/Hayabusa-2)

The Hayabusa-2 mission (Figure 23) was considered as Category-II¹⁷ for the outbound journey with particular attention needed to “**avoid impact with Mars under all mission scenarios**”, and as Category-Vu¹⁸ during the inbound journey, corresponding to “unrestricted Earth return”. Figure 24 shows orbits of the Earth (blue line), Hayabusa-2 (green line), asteroid 1999JU3 or Ryugu (purple line), and Mars (red line). The orbits of the spacecraft and Mars are not so close to each other, but of course there are relatively higher chances of encounter compared to other planets.

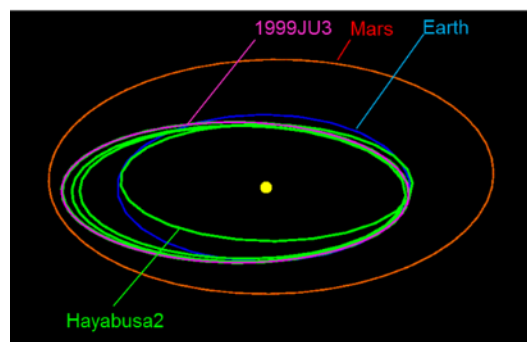


Figure 24 – Orbits of the Earth, Hayabusa-2, and 1999JU3 (Ryugu).

¹⁶ This section is based on a presentation provided by Dr. Toshihiro Chujo from ISAS/JAXA.

¹⁷ Category II: Flyby, Orbiter, Lander: Comets; Carbonaceous Chondrite Asteroids; Jupiter; Saturn; Uranus;

Neptune; Pluto/Charon; Kuiper-Belt Objects; others TBD.

¹⁸ Category V: Any Earth-return mission. “Restricted Earth return”: Mars; Europa; others TBD; “Unrestricted Earth return”: Moon; others TBD.

Ryugu is far enough (i.e., has zero collision probability) both from the Earth and Mars.

HOW TO PROVE COMPLIANCE?

The required probability value differs, depending on missions. In the case of the Hayabusa-2 mission, the requirement was that the probability of impact of the spacecraft with Mars under all possible mission scenarios must be less than 10^{-4} in the 50-year period after its launch.

The difficulty is to prove it quantitatively. Generally speaking, accidental impact is only realized in a sequence of spacecraft failure and passive motion of spacecraft that lost control after the failure. Therefore, one needs to compute the product of the failure probability by the impact probability after failure. The first term depends on the system failure rate and the meteoroid kill rate. The second term depends on the mission under consideration or its trajectory design, as it is strongly related to orbital dynamics.

FAILURE PROBABILITY: SPACECRAFT SYSTEM FAILURE RATE

Estimating the spacecraft system failure rate requires referring to the reliability of subsystems: What is the most critical component? Which component is the dominant factor? How to estimate the failure probability?

These questions illustrate how important the design philosophy of the spacecraft is.

For Hayabusa-2 (Figure 25), the design philosophy was set such that any bus subsystem (i.e., data handling, communication, power management, thermal control, attitude, etc.) of the

spacecraft have higher reliability than the IES (Ion Engine System). In other words, the IES was considered to be the critical component and the failure of the IES must be considered first.

The relationship between reliability R and failure rate λ is usually represented by:

$$R = \exp(-\lambda t)$$

This relationship means that the reliability decreases exponentially as time passes. The parameter λ is determined by specification of a subsystem. For Hayabusa-2, the IES (the critical component) was designed in such a way that 3 out of 4 thrusters (75 %) must remain in good condition for 6 years.

Then, with $R = 0.75$, $t = 6$ [yr], one can derive $\lambda = 1.3 \times 10^{-4}$ [1/day]. However, this is just a pure reliability evaluation of the IES itself. For the reliability related to Mars impact, which is represented as R_{MI} , we have to consider the fact that even if system failure occurs, an impact with Mars can be avoided as long as at least one of the four IES thrusters is operative.

$$R_{MI}(t) = 1 - \{1 - \exp(-\lambda t)\}^4 - f_{bus}$$

$$R_{MI}(t) \approx 1 - \{1 - \exp(-\lambda t)\}^4$$

where $R_{MI}(t)$ represents the Reliability related to Mars impact and f_{bus} is the failure probability due to bus subsystem malfunction. R_{MI} is the total probability 1 minus the probability that every thruster is dead and minus failure probability of other subsystems. As is mentioned, the critical component is the IES and f_{bus} can be regarded as negligible in this case.

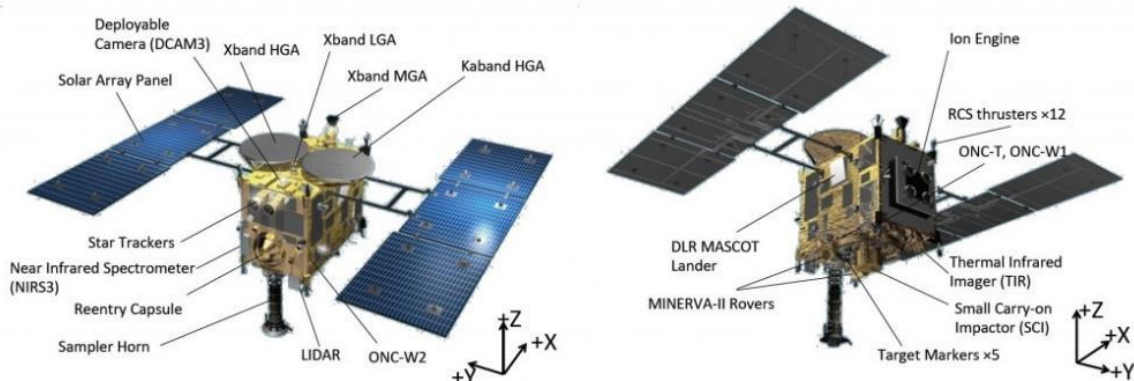


Figure 25 – Hayabusa-2 (Credit : JAXA)

FAILURE PROBABILITY: METEOROID KILL RATE

Hypervelocity impacts by meteoroids are unavoidable. Therefore, the questions to address are: What are scenarios of fatal meteoroid impact consequences? What is the minimum mass of meteoroid that realizes fatal impact? How to estimate the meteoroid kill rate? To analyze these, we should list up all possible scenarios first.

In the case of Hayabusa-2, we defined three scenarios. They are disruption of all the 4 IES grids, penetration of 1 of the 12 RCS thrusters, and dark current by impact intruding to spacecraft circuits through the honeycomb panel.

For each case, by considering the dimension and also attachment to the main body, probability of meteoroid impact can be calculated geometrically. Next, from the structural strength which is mainly thickness of the components, minimum mass of meteoroid that destroys them is estimated.

Finally, from these two analyses and information of interplanetary meteoroid flux, we can calculate the resulting probability of the kill rate for each component. In this case, the probability of the case three is much higher than other two.

The Table 8 below shows the analysis in the case of Hayabusa-2.

The dark current scenario overwhelms other effects so that the total meteoroid impact kill rate is approximated as $\phi_{total} \approx 0.026 [yr^{-1}]$.

Thus, the total probability q of a failure occurring between t_1 and t_2 is given by:

$$q(t_1, t_2) = R_{MI}(t_1) - R_{MI}(t_2) + \phi_{total}(t_2 - t_1)$$

Important to remember are: the design philosophy of the spacecraft; the critical system component for failure; the reliability function; the critical component for meteoroid kill; and, the kill rate analysis referring to component properties.

	(i)	(ii)	(iii)
Scenario	Disruption of all the 4 IES grids	Penetration of 1 of the 12 RCS thrusters	Dark current by impact intruding to spacecraft circuits through the honeycomb panel
Component dimension	150 mm diameter	64×64 mm	1.6×1.0×1.25 m
Minimum mass of meteoroid	8×10^{-6} g	1×10^{-4} g	1×10^{-3} g
Kill rate	2.5×10^{-7} [yr ⁻¹]	2.1×10^{-4} [yr ⁻¹]	2.6×10^{-2} [yr ⁻¹]

Table 8 – Meteoroid kill rate in three scenarios for Hayabusa-2

IMPACT PROBABILITY AFTER FAILURE

The total probability P_{total} of Mars impact is represented by:

$$P_{total} = \int p q dt$$

where p is the Mars impact probability after spacecraft failure. In other words, it is the probability that the spacecraft out of control reaches Mars on a ballistic trajectory. Here ballistic indicates that the spacecraft flies according to external force (Figure 26).

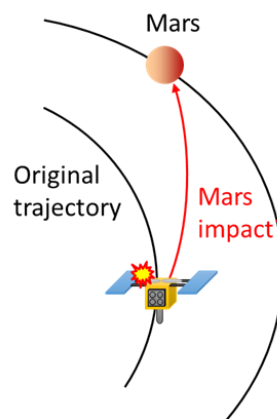


Figure 26 – Spacecraft impact on Mars on a ballistic trajectory.

This problem relates to orbital dynamics. The question is: What is the probability to accidentally enter a trajectory that leads to Mars?

In the case of a Mars orbiter, the relevant parameters are the gravity of Mars (as well as the Sun), and perhaps the air drag. For an interplanetary probe, one must consider the gravity of the Sun and possibly the gravity of the Earth and other planets. That will accelerate the spacecraft and change the trajectory significantly, which is really a complex problem. We focus here on the case of interplanetary probes using the example of Hayabusa-2.

In Figure 27, the Mars orbit is in red, the Earth orbit in blue and Hayabusa-2 orbits in the nominal and backup windows in green.

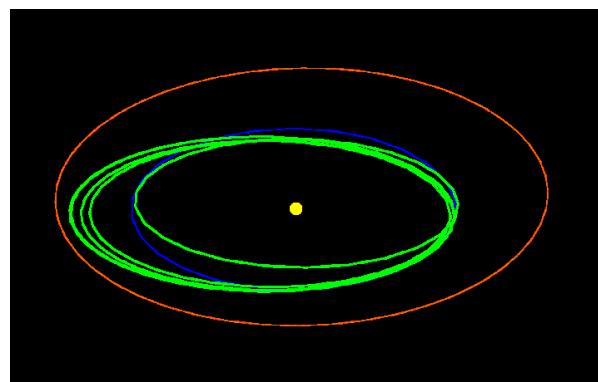
A rough estimate of the impact probability can be obtained by looking at the minimum distance to Mars. The minimum distance between the Hayabusa-2 spacecraft and Mars is 14×10^6 km for the mission trajectory of the nominal window (which was actually used). For the backup windows, it was 14×10^6 km and 5×10^6 km, respectively.

Since the trajectory guidance accuracy is of a few hundred kilometers at worst, an impact of the spacecraft with Mars is unlikely, due to uncertainties in trajectory determination.

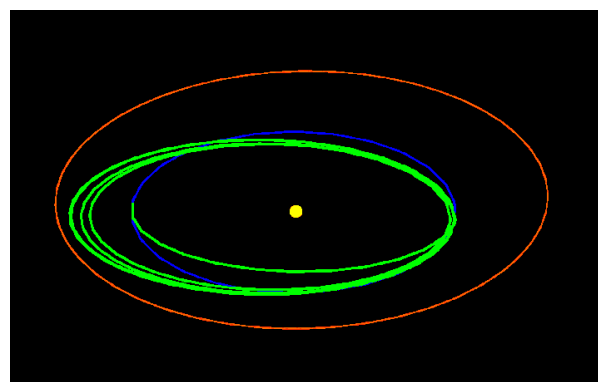
Next, the guidance error should be evaluated. As already mentioned, impact to Mars is unlikely, and that means that ΔV assistance is necessary to reach Mars. As a typical cause of ΔV , guidance error is investigated here. Here, the operation frequency is important. For Hayabusa-2, the trajectory navigation and guidance is performed using one-week cycles and orbit determination is updated no later than one month. Maximum acceleration produced by the IES is ~ 100 m/s per month. The attitude to operate the IES is constrained to within 10° from the Sun direction. Therefore, in the worst case, the maximum guidance error for one month¹⁹ is given by:

$$100 \text{ [m/s]} \times 10 \text{ [deg.]} \times \pi/180 \approx 20 \text{ [m/s]}$$

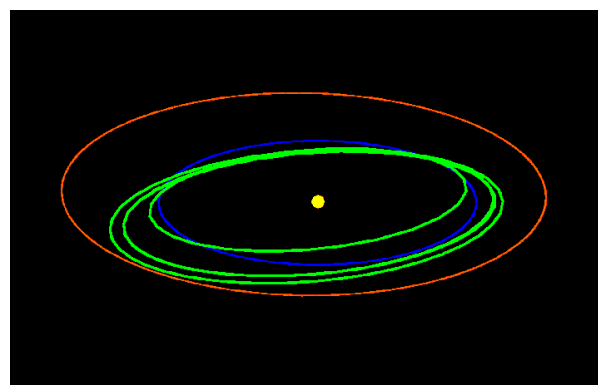
¹⁹ The ΔV produced by the RCS is negligible.



End-to-end mission trajectory for Nominal Window[N]
Minimum Dist. = 0.091AU (= 14 Mkm)



End-to-end mission trajectory for Backup Window1[BU1]
Minimum Dist. = 0.091AU (= 14 Mkm)



End-to-end mission trajectory for Backup Window2[BU2]
Minimum Dist. = 0.032AU (= 5 Mkm)

Figure 27 – Minimum distance between Hayabusa-2 and Mars for the nominal and backup windows

The left term is the product of the maximum ΔV by the IES by the maximum angle error. The result on the right is the worst ΔV in the undesired direction (Figure 28). Whether this estimated worst ΔV may realize Mars impact

or not can be calculated using the method of Lambert's problem (see below).

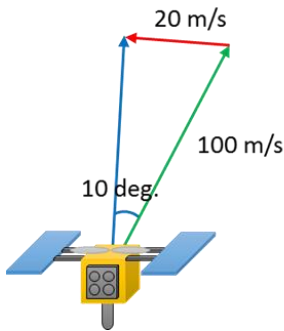


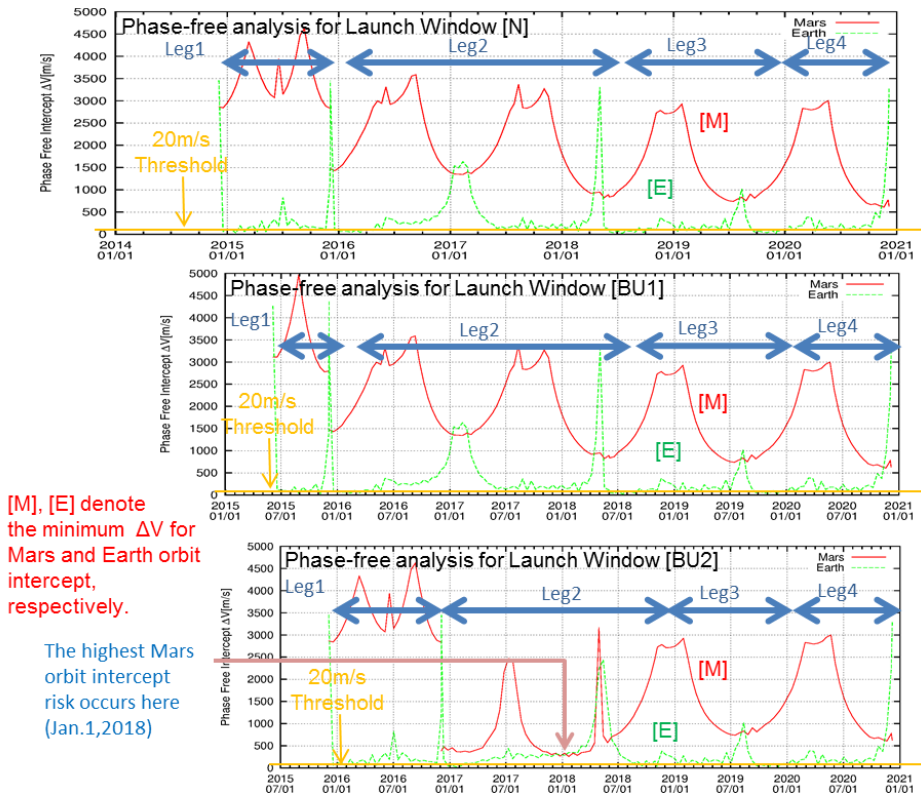
Figure 28 – ΔV generated by guidance error.

Based on the Lambert's problem, the minimum departure ΔV to intercept the Mars orbit – ignoring its orbital position – can be evaluated.

The results are shown in Figure 29 for the three launch windows. Horizontal axis and vertical axis indicate time and required ΔV for interception. Leg 1, 2, 3, 4 just mean phases of the mission. The threshold of 20 m/s, maximum ΔV produced by guidance error, is displayed by yellow lines. On the other hand, required

ΔV to reach Mars is shown with red lines. Discontinuous points are observed between Leg 1 and 2, because the Hayabusa-2 spacecraft performs the Earth swing-by to get gravity assist. The value of the red line is much larger than the yellow line, especially in the nominal window and the backup window 1. Smaller values are observed in the backup 2, where the highest Mars orbit intercept risk occurs, but still, required ΔV exceeds the produced ΔV .

This result indicates that the probability of Mars direct impact is practically zero. However, we also investigated ΔV to reach the Earth based on Lambert's problem. Since Hayabusa-2 once come back to the nearby region to the Earth for swing-by, the required ΔV is much smaller than Mars. This implies that the probability to impact Mars via Earth gravity assist is not negligible and should be discussed additionally. It is beyond Kepler orbit and Lambert's problem, so we had to cope with the problem in another way.



[M], [E] denote the minimum ΔV for Mars and Earth orbit intercept, respectively.

The highest Mars orbit intercept risk occurs here (Jan.1,2018)

Figure 29 - Results of rough estimation of direct impact to Mars

Transfer trajectory to Mars dealt with as a Lambert’s problem

Lambert’s problem assumes a spacecraft under the influence of a central gravitational force traveling from point P_1 to a point P_2 in a time T (Figure 30). Then the ballistic transfer trajectory can be solved, assuming a two-body problem with Kepler orbit. If the gravity of the Sun is dominant, it is useful. r_1 , r_2 , and T being given, the initial velocity v_1 is resolved. Then the impulse ΔV necessary to reach P_2 can be solved numerically.

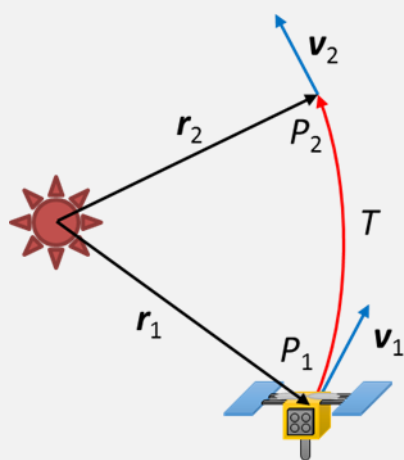


Figure 30 – Graphic representation of Lambert’s problem.

In the Hayabusa-2 mission, the probability of direct impact to Mars is zero, but the probability of getting gravity assist of the Earth is not

zero. This happens because the original trajectory aims at the Earth swing-by. Thus, the original trajectory design is also important.

If the spacecraft may pass near the Earth or other planets such as Venus, it should be taken into account in the probability analysis. Therefore, we used Monte-Carlo simulation, which was thought to be a suitable method because the dynamics of swing-by is really sensitive to initial conditions, or approach way to the Earth, and that makes large dispersion of probability. We think that Monte-Carlo simulation was better than other possible simpler methods. Figure 31 shows a flow chart of impact probability analysis, both for direct impact cases and swing-by cases for comparison.

Hereunder is the algorithm used for the Hayabusa-2 mission. It consists of two steps. The first step is Monte-Carlo propagation and the second step is statistical processing, which is necessary because the number of cases in the Monte-Carlo simulation is practically limited considering the computational cost. The probability is evaluated by the time integral of the probability function, so ideally we have to consider every single case of timing when the system failure occurs, which is infinite. In practice, however, we discretized the time every one month, and performed Monte-Carlo simulation starting each day.

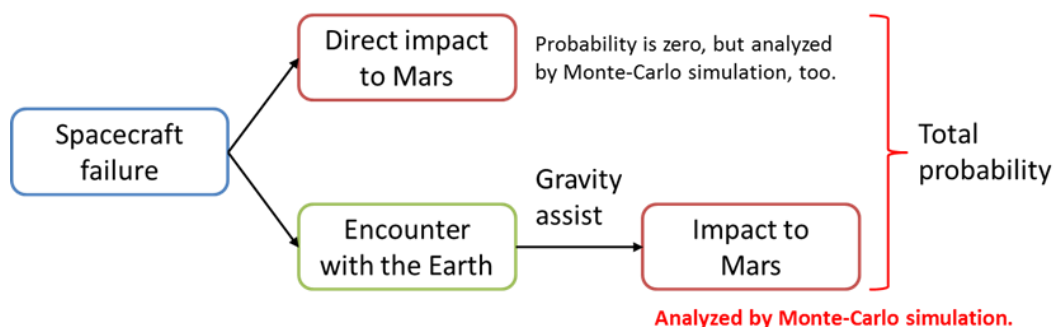


Figure 31 – Flow chart of impact probability analysis.

For each starting day, we defined the number of cases as 1,000, and propagation time was set to be 50 years after the launch as is required by the Planetary Protection Policy (Figure 32).

For initial deviations, we gave Gaussian random numbers with variation according to accuracy of orbit determination and guidance error. In the computation, we took gravity influence of all the planets and the Sun using

ephemeris. Also, we should not forget that the roughness caused by the approximation of discretization is calculated as ΔV times t , which is around 50,000 km.

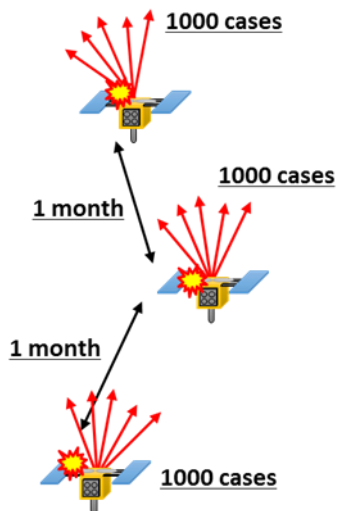


Figure 32 – Representation of discretized Monte-Carlo simulation.

Judgement of swing-by cases or no swing-by cases, or direct impact cases, was evaluated by the distance from the Earth to the intersection point of the spacecraft on the B-plane of the Earth. B-plane is a plane perpendicular to the orbital motion around the Sun. We defined the threshold as 1 million km. If the spacecraft passes near the Earth by 1 million km even once, the case is defined as a "swing-by case", and otherwise, "no swing-by case". For example, in our analysis, for cases where system failure occurs on January 1, 2016, 915 cases out of 1,000 cases were defined as no swing-by cases and other 85 cases were defined as swing-by cases. For both of these, we prepared different statistic processing algorithms.

The algorithm for "no swing-by cases" is briefly described. First, we investigated every intersection point on the Mars B-plane. Figure 33 shows the actual results for the one group of cases. In the figure, the position of the Sun is fixed and therefore the position of Mars expressed by red points moves a bit according to its orbital motion. Blue plots are intersection points of the spacecraft, and some dispersion originating from the initial deviations can be observed. Among these, no case reached the Mars positions, and this is why we have to do

statistic processing. Figure 34 shows the deviation of the plots on Figure 33. It looks it can be approximated by Gaussian distribution by fitting calculation, which is shown by the red line. This extends the probability to the infinity distance. Then the probability of Mars impact can be calculated to be non-zero. Of course, it's actually almost zero.

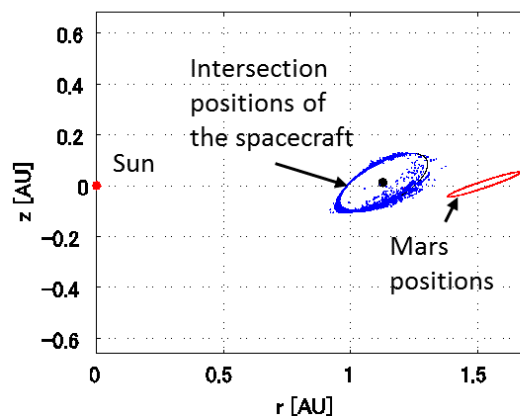


Figure 33 - Example of intersection points on Mars B-plane.

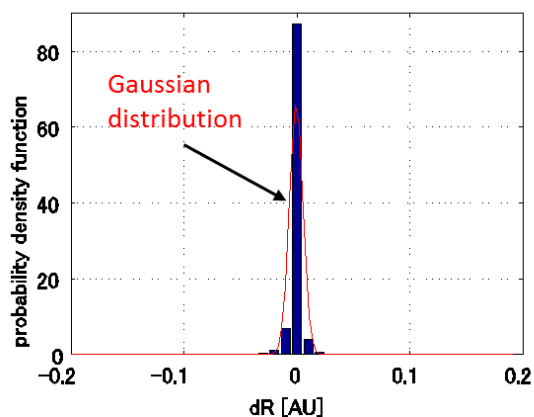


Figure 34 - Deviation of the plots on Figure 33

Next, the algorithm for "swing-by cases" is briefly described. It is actually a bit complex, so here I show the approach just simply. First, transfer trajectory after gravity assist by the Earth to Mars is made by Lambert's problem in the same way. Second, a keyhole on the Earth B-plane that connects to the transfer trajectory is searched (Figure 35). The ratio of the area of this key hole to the area of a circle with the radius of 1 million km is one reference for the probability. Then statistical processing is conducted, and finally the probability of Mars impact is derived.

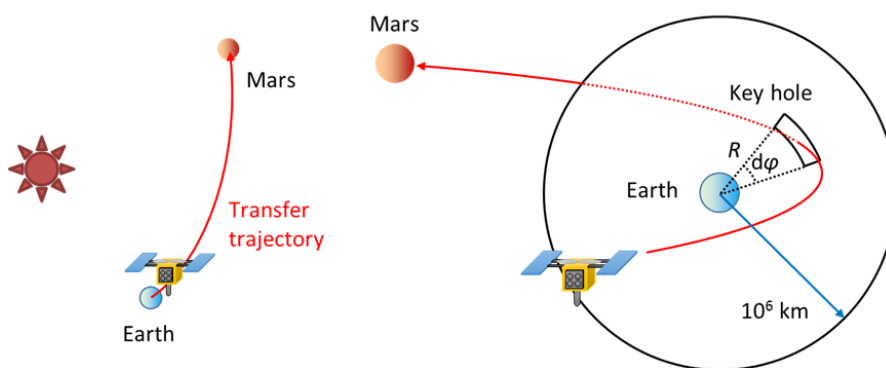


Figure 35 - Representation of the algorithm for “swing-by cases”.

Finally, the results of these calculations for Hayabusa-2 in the case of the nominal window is given in the Table 9 below. For each trajectory leg, the third column is the total probability of no swing-by cases, and fourth column shows the impact probability of swing-by cases. The fifth column shows the failure probability and the right column gives the resulting total probability. Zero values in the Table correspond to values less than 10^{-50} .

Total probability for backup window 1 and 2 are 8.0×10^{-12} and 5.6×10^{-7} , respectively. Therefore, the Hayabusa-2 mission satisfies the COSPAR requirement that the Mars impact probability should be less than 10^{-4} for 50 years after launch for all mission scenarios.

Trajectory Leg	IES ΔV /RCS ΔV	Impact probability (no swing-by) $\int p_{no_swby} dt$	Impact probability (swing-by) $\int p_{swby} dt$	Failure probability	Total probability $\int (p_{no_swby} + p_{swby}) q dt$
Injection	No/No	1.0e-14	1.9e-12	2.1e-3	4.1e-15
Earth to Earth	Yes/Yes	4.1e-13	1.9e-11	2.4e-2	4.1e-14
Earth to Asteroid	Yes/Yes	0	3.7e-11	6.8e-2	8.8e-14
Asteroid Proximity	No/No	0	0	4.1e-2	0
Asteroid to Earth	Yes/Yes	0	3.4e-9	2.8e-2	8.0e-12
TOTAL Probability		4.1e-13	3.4e-9	1.6e-1	8.1e-12

Table 9 – Impact probability for the nominal window.

SUMMARY

- Spacecraft failure rate and probability of impact after the failure have been respectively calculated to prove the probability of Mars impact quantitatively.
- The design philosophy of the spacecraft and reliability of subsystem component are important factors when considering system failure.
- Making ballistic trajectory to Mars and estimating probability to enter it accidentally

is the first step for estimation of impact probability.

- For missions where the spacecraft may be assisted by the Earth gravity, it may be necessary to analyze the probability using Monte-Carlo simulation.

Chapter 2. Case Studies

This chapter presents a number of case studies illustrating the various categories introduced in Chapter 1, from Category II to Category V.

PLANETARY PROTECTION CATEGORY II²⁰

PLANETARY PROTECTION CATEGORY II DESCRIPTION

All types of missions (gravity assist, orbiter, lander) to a target body where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote²¹ chance that contamination carried by a spacecraft could compromise future investigations.

Applicability: Moon (with organic inventory), Venus, comets, carbonaceous chondrite asteroids, Jupiter, Saturn, Uranus, Neptune, Ganymede*, Callisto, Titan*, Triton*, Pluto/Charon*, Ceres, KBO >½ size of Pluto*, KBO <½ size of Pluto.

*The mission-specific assignment of these bodies to Category II must be supported by an analysis of the remote potential for contamination of a liquid-water environment that may exist beneath their surfaces, addressing both the existence of such environments and the prospect of accessing them.

CASE STUDY FOR PLANETARY PROTECTION CATEGORY II: BEPICOLOMBO

- Target body: Mercury (Category I);
- Propulsion: Electrical for cruise (transfer module jettisoned before arrival at Mercury), chemical around Mercury;
- Transfer: Multiple Earth, Venus (Category II), and Mercury gravity assists → mission level Category II.

REQUIREMENTS FOR CASE STUDY

Requirements for Category II missions are limited to documentation:

- Planetary Protection Plan → due at CDR (draft at PDR useful);
- Pre-Launch, Post-Launch, and End-of-Mission Reports at the respective milestones.

However, ...

...all missions leaving Earth orbit must demonstrate compliance with impact probabilities or bioburden limits for Mars and probability of contamination limits for Europa & Enceladus.

- For the specific case study of Bepi-Colombo there is no plausible trajectory leading to the outer solar system;
- The probability of impact on Mars by any element not assembled and maintained in ISO level 8 conditions (i.e. launcher upper stage) shall be $\leq 1 \times 10^{-4}$ for the first 50 years after launch;
- The probability of impact on Mars by any part of a spacecraft assembled and maintained in ISO level 8 cleanrooms, or better, is $\leq 1 \times 10^{-2}$ for the first 20 years after launch, and $\leq 5 \times 10^{-2}$ for the time period from 20 to 50 years after launch.

THINGS TO REMEMBER

- Requirements for Category II missions are limited to documentation and reviews;
- Additional analysis and consequences on flight system design and mission operation can be necessary in particular for missions crossing the orbit of Mars, going to the outer solar system, or performing gravity assist maneuvers;
- Probability of impact requirements can have an effect on the trajectory design, the

²⁰ This section was provided by Dr. Gerhard Kminek.

²¹ Implies the absence of environments where terrestrial organisms could survive and replicate, or a very low

likelihood of transfer to environments where terrestrial organisms could survive and replicate.

delta-v budget (re-targeting), and spacecraft design (e.g., location of tanks, additional micrometeoroid protection);

- To accommodate these effects, have a first trajectory analysis ready for the PDR;
- This first trajectory analysis should not be too simplistic – otherwise late changes in the spacecraft design or operation might become necessary;

- Ensure good interface with launcher system for upper stage impact analysis;
- All activities necessary to perform a probability of impact analysis are interdisciplinary and require the interactions between different engineering disciplines!

PLANETARY PROTECTION CATEGORY III²²

PLANETARY PROTECTION CATEGORY III DESCRIPTION

Flyby (i.e., gravity assist) and orbiter missions to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant²³ chance of contamination which could compromise future investigations.

Applicability: Mars, Europa, Enceladus.

However, if an orbiter mission is looking for life, the mission will have to meet requirements for a life detection mission (i.e., avoid compromising the life detection measurement).

CASE STUDY FOR PLANETARY PROTECTION CATEGORY III: EXOMARS TRACE GAS ORBITER (TGO)

- Target body: Mars.
- Propulsion: Chemical.
- Transfer: Deterministic Deep Space Maneuver (DSM) with several 100 m/s and stochastic Trajectory Correction Maneuvers (TCMs) with several m/s.
- Orbit acquisition: Mars Orbit Insertion (MOI) maneuver with several 100 m/s and aerobraking.
- Final orbit: 400x400 km, 373:30 repeat pattern.

REQUIREMENTS FOR CASE STUDY

Launcher upper stage

The probability of impact on Mars by any element not assembled and maintained in ISO level 8 conditions shall be $\leq 1 \times 10^{-4}$ for the first 50 years after launch

Note: This requirement also applies if a launch service is provided to another customer, e.g., launch of the Emirates Mars Mission (EMM) on H-IIA.

Spacecraft

One of the following conditions shall be met:

- The probability of impact on Mars by any part of a spacecraft assembled and maintained in ISO level 8 cleanrooms, or better, is $\leq 1 \times 10^{-2}$ for the first 20 years after launch, and $\leq 5 \times 10^{-2}$ for the time period from 20 to 50 years after launch (e.g., Mars Express, TGO).
- The total bioburden of the spacecraft, including surface, mated, and encapsulated bioburden, is $\leq 5 \times 10^5$ bacterial spores (e.g., MRO, Maven).

IMPLEMENTATION OF REQUIREMENTS FOR CASE STUDY

Launcher upper stage

The probability of impact on Mars by any element not assembled and maintained in ISO level 8 conditions shall be $\leq 1 \times 10^{-4}$ for the first 50 years after launch*.

*Relevant requirement needs to be reflected in Launcher Interface Requirements.

Trajectory analysis based on Monte Carlo method to achieve a one-sided 99% level-of-confidence.

- Trajectory analysis covers all reference trajectories for the launch window (i.e., > 1).
- Number of Monte Carlo runs depends on the detected number of impacts (iterative).

Analysis includes:

- Gravity potential of Earth and Mars and 3rd body perturbation by Sun, Moon, Jupiter and Saturn;
- Solar radiation pressure (SRP) with uncontrolled attitude;
- Propellant blow-down as directed contribution, outgassing as spherical contribution;
- In case there is a maneuver of the upper stage after the release of the spacecraft

²² This section was provided by Dr. Gerhard Kmínek.

²³ Implies the presence of environments where terrestrial organisms could survive and replicate, and some likelihood of transfer to those places by a plausible mechanism.

(e.g., for Breeze-M and Centaur), the reliability of this maneuver (from flight records) has to be part of the overall analysis;

- In case impact probability is too high → increase launch bias away from Mars (effect on delta-v budget for spacecraft).

Spacecraft

1. Analyze the stability of the final science orbit → stable for > 50 years ✓

- Numerical propagation of orbit with atmospheric variation (driven by solar cycle); evaluate right ballistic parameter and proper parameters from the atmospheric model

2. Analyze the impact probability of the spacecraft before the DSM → no impact > 50 years ✓

- Monte Carlo method to achieve a one-sided 99% level-of-confidence;
- Necessary input is the launcher dispersion matrix (injection conditions);
- Gravity potential of Earth;
- 3rd body perturbation by Sun, Moon, Jupiter and Saturn;
- Solar radiation pressure (SRP) with controlled and un-controlled attitude.

3. Analyze the impact probability between the DSM and reaching the final science orbit

- Assume trajectory impact probability of ‘1’ after DSM (conservative);
- Reliability of the flight hardware necessary to control the spacecraft and reliability of operation;
- Atmospheric variation for Mars aerobraking phase; ignore chance for recovery (conservative);
- Micrometeoroid impact and effect analysis (details next).

Probability of impact: $P_{\text{HW failure}} + P_{\text{OP failure}} + P_{\text{meteoroid kill}} \leq 1 \times 10^{-2}$

1. Micrometeoroid model definition

- Selection of micrometeoroid flux model (e.g., Grün), velocity distribution (e.g., 20 km/s), micrometeoroid density (e.g., 2.5

g/cm³), and average impact angle (e.g., 45°).

2. Analysis of consequences

- Selection of critical units necessary to control spacecraft (see reliability analysis);
- Assess protection based on presence of MLI, panels, honeycomb panels, etc. in terms of equivalent thickness; take into account view factors;
- Assess protection based on distances between different elements to select use of proper ballistic limit equation (BLE from IADC);
- Assess the failure modes of critical hardware;
- Typical problematic hardware, e.g., tanks, star trackers, propulsion lines, UHF RFDN waveguides.

The approach used for the TGO is conservative in many ways but also easier to evaluate.

This approach can be used for orbiter missions and for cruise stages delivering a lander.

In case this approach is not sufficient to demonstrate compliance with the probability of impact requirements, other approaches could be used (but do not guarantee compliance):

- Replacing the fixed micrometeoroid velocity with a velocity distribution (e.g., Taylor);
- Replacing the trajectory impact probability value of 1 during part of the cruise phase with a value based on a trajectory analysis and allowing recovery maneuvers;
- Allowing recovery maneuvers during aerobraking.

THINGS TO REMEMBER

- Probability of impact requirements can have an effect on the qualification of hardware (e.g., solar arrays for aerobraking), the trajectory design, the ΔV budget (re-targeting), and spacecraft design (e.g., location of tanks, additional micrometeoroid protection);
- To accommodate these effects, have a first analysis ready for the PDR;

- This first analysis should not be too simplistic – otherwise late changes in the spacecraft design or operation might become necessary;
- There is a trade-off in the aerobraking design between more gentle and longer aerobraking (negative for micrometeoroid effects and reliability) and more aggressive and shorter aerobraking (negative for hardware qualification and operation);
- Ensure good interface with launcher system for upper stage impact analysis;
- All activities necessary to perform a probability of impact analysis are interdisciplinary and require the interactions between different engineering disciplines!

PLANETARY PROTECTION CATEGORY IVA – INSIGHT²⁴

CATEGORY IVA CASE STUDY OVERVIEW

For Category IVa missions to Mars (as opposed to Category I, II and III) the main feature is bioburden control of the spacecraft hardware prior to launch, to achieve a particular microbial cleanliness level. Similar approaches would be valid for lander missions to Europa or Enceladus, depending on mission design, but would potentially also include some Category III missions to those targets, where indications are that the required contamination avoidance cannot be met by simply avoiding impact at the target body at the required probability level.

PLANETARY PROTECTION CATEGORY IVA DESCRIPTION

Formally, Category IV missions are lander missions to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant chance of contamination which could compromise future investigations. As noted, based on COSPAR policy at the time of writing, the applicability is to Mars, Europa and Enceladus.

For Europa, Enceladus compliance with the planetary protection policy is by the Project demonstrating that the probability of inadvertent contamination of a body of liquid water is limited to less than 1×10^{-4} per mission. The microbial cleanliness level is one input factor in a probability of contamination calculation.

However, for Mars missions, the policy compliance is demonstrated by achieving a specific microbial cleanliness level alone, based on one out of three subcategories (a, b, c), as described in Chapter 1, “Planetary Protection Requirements”.

While for missions to outer solar system targets, “spores” may not be the relevant measure: some other organism may be the relevant resistant organism to be used for spacecraft bioburden accounting. However, the standard

spore assay is a robust assessment of spacecraft microbial cleanliness, and can often be related to the abundance of other organisms (see e.g., the SSB report on the prevention of the forward contamination of Europa), hence the InSight case study is relevant in the context of a bioburden controlled mission to other targets as well as Mars.

CASE STUDY FOR PLANETARY PROTECTION CATEGORY IVA – INSIGHT

INSIGHT MISSION OVERVIEW

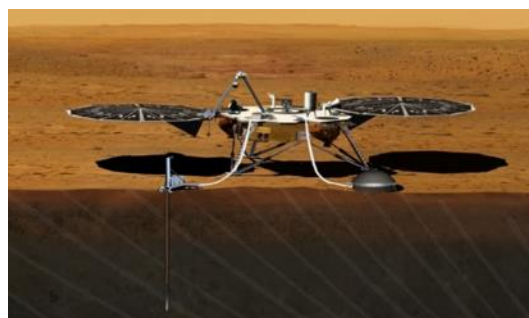


Figure 36 – Illustration of the InSight mission deployed on the Martian surface (Courtesy NASA/JPL)

The InSight mission (Interior Exploration using Seismic Investigations, Geodesy and Heat Transport) was launched on May 5, 2018 and landed on November 26, 2018.

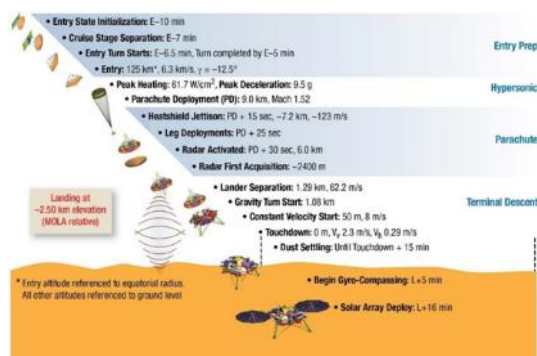


Figure 37 – Entry descent and landing timeline for the InSight mission (Courtesy NASA/JPL)

Key information on the mission characteristics are as follows:

- Target body: Mars;
- Mars Lander (360 kg) based on Phoenix heritage (Figure 36);

²⁴ This section is based on a presentation by Dr. J Andy Spry, SETI Institute.

- Science instruments contributed by CNES (SEIS) and DLR (HP3);
- Launch Window May 5, 2018, on ATLAS V401 from Vandenberg AFB in California;
- 6.5-month cruise, type 1 trajectory, direct entry (Figure 37 and Figure 38).

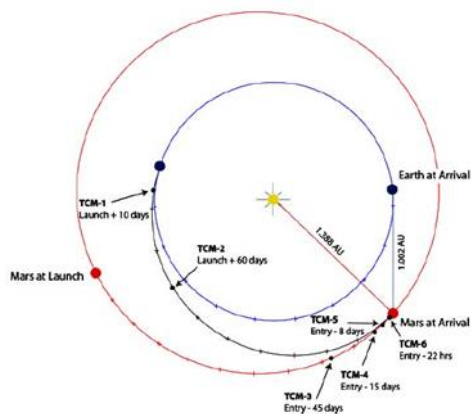


Figure 38 – Interplanetary trajectory for the InSight mission (courtesy NASA/JPL)

The mission is scheduled to perform one Martian year of science on the surface, to (i) Understand the formation and evolution of terrestrial planets through investigation of the interior structure and processes of Mars, and (ii) to determine the present level of tectonic activity and meteorite impact rate on Mars.

REQUIREMENTS FOR THE INSIGHT MISSION

As a Mars lander mission without life detection instruments, the InSight mission has been designated PP Category IVa by the NASA PPO. In accordance with the requirements stated in NASA Procedural Requirements document NPR8020.12 for this category and type of mission, the InSight Project is required to comply with the following:

Bioburden requirements:

- At launch accounting of $\leq 5 \times 10^5$ total spores, $\leq 3 \times 10^5$ total spores on planned landed hardware and mean exposed surface density of < 300 spores/m².

Cleanliness requirements:

- Assembly and testing in ISO 8 (or better) cleanroom environments.

Recontamination avoidance requirements:

- Launch recontamination not to exceed at launch bioburden requirements.

Organic inventory requirements:

- Archiving of samples of at least 50grams of each organic material type for which more than 25 kg is transported to Mars.
- Documentation of organic materials for which are present on the spacecraft in quantities of ≥ 1 kg.

Probability of Impact requirements:

- Launch vehicle Mars avoidance of less than 1×10^{-4} for 50 years after launch, and probability of a non-nominal impact of Mars by the spacecraft due to cruise phase failure shall be $\leq 1 \times 10^{-2}$.

In addition, in following current established practice and after consultation to obtain best available advice, the NASA Planetary Protection Officer imposed the following additional planetary protection requirements:

- The average internal (behind HEPA or tortuous path) bioburden was required to be $\leq 1,000$ spores/m².
- The mole shall be unpowered and cease operations immediately if the tether to the lander breaks during surface operations.
- Ice shall not be present within reach of HP3 instrument's mole (to be demonstrated thermodynamically by pre-launch analysis).
- The mole shall not generate a thin liquid film as a result of operations (insufficient to transport a 50nm particle, to be demonstrated by pre-launch analysis).
- Planetary Protection Landing Site Review was required.
- Project shall utilize NASA PPO-provided "new" heat microbial reduction specifications which provide expanded implementation options (e.g., no humidity constraints, credit for manufacturing processing), as superseding the "old" specifications in the present NPR8020.12D document
- PP requirements to be captured into the Level 2 Project System Requirements Document [first JPL Project to capture all PP

requirements into the Dynamic Object Oriented Requirements (DOORS) V&V tool].

- All Level 2 and 3 requirements are under Project Change Control Board management.

The early entries in these additional requirements are associated with the project demonstrating that the mission would not cause transgression of the parameters for a Category IVc mission that would have required a higher level of cleanliness.

In the latter part of the list, the requirements are to ensure incorporation of planetary protection into system engineering best practice in the implementation and V&V aspects of the requirements.

IMPLEMENTATION OF REQUIREMENTS

BIOBURDEN ACCOUNTING

The project adopted the standard practice for Martian Category IVa missions by allocating

the Maximum Accounted Bioburden Requirement of 500,000 spores into a budget (Table 10), and managing the bioburden budget using a PPEL (Planetary Protection Equipment List), analogous to the spacecraft MEL (Mass Equipment List) but with cleanliness heritage, assayed and accounted bioburden and surface and volume entries, rather than mass (see excerpted illustration of a PPEL in Figure 39 below).

	spores
Lander	
PHX Landed Hardware (minus parachute)	32,000
Parachute	32,000
New External Lander Hardware and LM Reserves	35,000
Payload	
SEIS	20,000
HP3	25,000
IDS	25,000
APSS	25,000
Impacting Hardware (PHX, actuals)	100,000
Launch Recontamination (MSL heritage value from Atlas V)	22,000
Project Held Reserves	180,000 (32%)

Table 10 – Illustration of the spore bioburden budget for the InSight Mission (courtesy NASA/JPL)

Figure 39 – Illustration of a NASA/JPL PPEL (Planetary Protection Equipment List) (Courtesy NASA/JPL)

For the (project specific) special analyses that were needed to demonstrate that the mission would not transgress planetary protection Category IVc constraints, the following work was performed:

LANDING SITE ANALYSIS

The Landing Site Characterization was to demonstrate that the selected Landing Site is NOT a Special Region (i.e., $A_w > 0.5$ AND $T > -18^\circ\text{C}$)²⁵.

²⁵ Note that the current Special Region lower temperature limit is -28°C , but at the time the InSight analyses were performed, the requirement was for $T > 18^\circ\text{C}$.

In addition, thermal modeling was performed to show the bounding sub-surface temperatures at the landing site over the full Mars year on the specific sols of the InSight Mission, including spacecraft activity, with the HP3 penetration phase expected around ~sol 67-100. A thermal model verification was also performed.

The analysis found that the short-lived temperature elevation of the subsurface above ambient due to HP3 hammering and thermal conductivity measurement activities is on the order of 10°-50°C. Mean subsurface temperatures at this site are -55°C, producing thermal elevations to ~0°C.

However, the regolith in the Elysium region is dry and ice-free, eliminating the possibility that HP3 heating would generate water activities in pore spaces that exceed the threshold for microbial activity. The maximum possible bulk water activity ($A_w = rh/100$) was estimated to be 0.09.

Hence, at the bulk scale, although the temperature regime during spacecraft operations may temporarily exceed one of the limits (temperature), the other (water activity) was not exceeded. So, in its native state, the landing site is not a special region, and with spacecraft operation, there is no chance for a spacecraft-induced special region to be created, hence the mission fulfils Category IVa requirements and is not a Category IVc mission.

SPECIAL ANALYSES – THIN FILM ANALYSIS

An analysis was also performed to understand the possibility that hydrous mineral composition in Martian soil capable of dehydration in the -55°-0°C range, following the activity of the mole, might liberate small amounts of water. This water could migrate, potentially entraining and carrying particles including terrestrial microbes, to environments where they may be able to replicate or to be transported to other more favorable environments.

The analysis performed is conservative, as it (a) accounts for MgSO₄ minerals not likely at

equatorial sites, and (b) factors in the maximum quantity of water that could be lost from total dehydration of those minerals.

The analysis found that a pulse of a small quantity of water due to the mole activity would generate 8 to 10 monolayer equivalents in the immediate vicinity of the mole. This would return to its equilibrium value of 2 monolayers within hours. Liberated water would flow under capillary action, spreading out in all directions, but the maximum film thickness is too small to entrain a 50 nm particle and is both a short-lived and small-distance phenomenon. This demonstrates that even at the micro-scale, the InSight mission does not transgress the special region limits that would make it a Category IVc mission.

PARTNER-CONTRIBUTED PAYLOAD MANAGEMENT

Another element of the InSight mission is the international partnership that exists for contribution of payload elements. The SEIS package is contributed from CNES, while the HP3 payload is contributed from DLR. For each of these elements, the InSight Project has PP Payload Implementation Plans, with the instrument providers then generating their own Institutional (i.e., CNES and DLR) Planetary Protection Plans.

The plans are integrated with flow down of PP requirements to L4 payloads, and implementation managed by frequent teleconferences and email exchanges (effective & efficient communication), addressing implementation approach questions and providing assay updates. PP is a topic area of discussion for HP3 and SEIS weekly teleconferences.

PP assay of interfaces, the hardware acceptance/certification process and status of bio-assays were on site activities at CNES and DLR with the InSight planetary protection engineer. Such integration across organizational and international boundaries is extremely desirable for smooth flow of operations, but needs early incorporation into inter-organizational planning (ICDs, contract agreements etc.).

IMPACT AVOIDANCE ANALYSIS

As with other missions, the InSight project has a requirement to ensure the probability of the (uncleaned) launch vehicle upper stage impacting Mars is less than 1.0×10^{-4} for 50 years after launch. Typically, this is achieved by biasing the injection aimpoint for launch away from Mars. Note that this requirement (for protection of Mars) would also be levied on missions to outer planets.

The analysis for InSight also included consideration of Centaur anomaly scenarios in the assessment, including; failure to separate, failure to perform CCAM, failure to implement blow-down.

The analysis for InSight also included consideration of Centaur anomaly scenarios in the assessment, including; failure to separate, failure to perform CCAM, failure to implement blow-down.

Based on the analysis, the following plan forward was approved as acceptable for demonstrating compliance with the requirement:

1. Design the biased aimpoints and CCAM attitude to ensure a minimum first-pass probability of impact less than 0.5×10^{-4} for all anomalous scenarios.
2. Design the blowdown attitude to ensure that the Mars encounter is sufficiently far away that the ΔV from the gravity assist is insufficient to place the Centaur on a 50-year resonant trajectory in the nominal scenario.
3. Perform 5,000-case, 50-year Monte Carlo propagations of the three anomalous scenarios to determine the Beta distribution shape parameters for the 50-year probabilities of impact.
4. Generate one million samples of each of the six Beta distributions representing the probability of an anomaly and the resulting 50-year probability of impact.
5. Combine the six million-sample sets and analytical probabilities to determine the distribution of the estimate of the total probability of impact.

At the time of writing, the approved methodology is being written up and submitted to a peer reviewed journal.

THINGS TO REMEMBER

For any bioburden controlled planetary protection implementation approach, including outer solar system Category IV missions (and Category III missions with bioburden requirements as a result of being unable to meet probability of impact avoidance requirements at an appropriate probability level), the implementation adopted by the InSight team provides a useful reference point.

As implemented, the InSight case study highlights the need to:

- Establish and maintain an end-to-end bioburden accounting approach.
- Pay attention to the details of provenance (manufacturing credit), inside/outside cleanliness, interfaces/environments, test activities, hardware processing, recontamination.
- Plan ahead for PP facilities needs and incorporation of PP into the ATLO flow.
- Identify and build in the time/resources necessary for high stringency cleanroom operation.

The InSight project highlights the benefit of good communication between PP implementers, hardware engineers, launch vehicles operations, project management and contributing hardware partners to the smooth implementation of planetary protection – so build in planetary protection in upfront, from the earliest planning stages of the project.

ACKNOWLEDGEMENTS

The PPOSS team would like to acknowledge the high level of cooperation of the InSight project team in the compilation of this material, in particular the support of the InSight Planetary Protection Lead, Dr. J. Nick Benardini.

PLANETARY PROTECTION CATEGORY IVB²⁶ – EXOMARS

The ExoMars program is an International Cooperation between the European Space Agency (ESA) and ROSCOSMOS (ROS) with instrument contributions of the National Aeronautics and Space Administration (NASA). ExoMars is composed by two different missions, the ExoMars 2016 mission, launched in 2016, and the ExoMars 2020, to be launched in 2020. Both Spacecrafts are launched with the Russian Proton-M rocket fitted with the Breeze-M upper stage from the Cosmodrome of Baikonur.

The objective of the ExoMars 2020 mission is to land the ESA Rover module and the Russian Landing Platform on Mars, which will perform exobiological and geological investigation on Mars surface. In particular, the technological and scientific objectives are the following:

- To demonstrate the main technologies of:
 - Entry, descent and landing (EDL) of a payload on the surface of Mars;
 - Surface mobility with a rover; and,
 - Access to the subsurface to acquire samples, sample acquisition, preparation, distribution and analysis
- Scientific objectives:
 - To search for signs of past and present life on Mars;
 - To investigate the water/geochemical environment as a function of depth in the shallow subsurface; and,
 - To characterize the surface environment.

The ExoMars 2020 spacecraft composite (SCC, Figure 40) is composed of elements provided by ESA and ROS. ESA provides the Carrier Module (CM) and Rover Module (RM, Figure 41) with the Survey and Analytical Payloads. ROS provides the Descent Module (DM) (without RM), which in turn composed of EDL/GNC System (with Parachute subsystem, Inertial Measurement Unit and Radar Doppler provided by ESA), Landing Platform

(including UHF TLC Subsystem and On Board Computer and software provided by ESA) and CM-DM separation adapter.

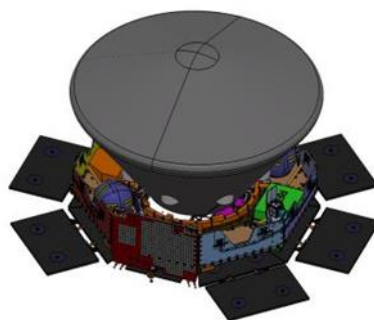


Figure 40 – SCC with deployed Solar Arrays (credit: TAS)



Figure 41 – The ExoMars Rover (credit: TAS)

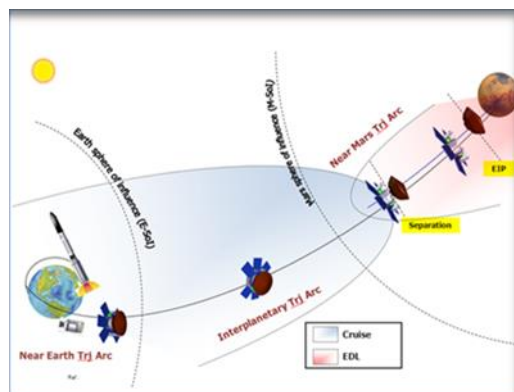


Figure 42 – Interplanetary transfer for ExoMars 2020 mission (credit: TAS)

The Launch window is 25 July 2020 (LPO)-13 August 2020 (LPC) and the Mission scenario is direct, ballistic Earth-to-Mars Transfer Trajectory landing on 19 March 2021 (Figure 42 and Figure 43). Currently, two different landing sites are under evaluation, Oxia Planum (18,16° N, 335,67° E) and Mawrth Vallis (LPO

²⁶ This section was contributed by Lic. Diana B. Margheritis (Thales Alenia Space).

22.42° N, 341.48° E; LPC 22,17° N, 341.99° E).

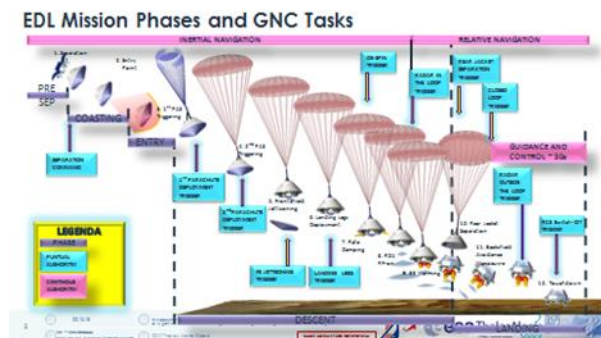


Figure 43 – Entry, Descent and Landing of ExoMars 2020 mission (credit: TAS)

PLANETARY PROTECTION CATEGORY IVB DESCRIPTION

“Category IV missions comprise certain types of missions (mostly probe and lander) to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant chance of contamination which could compromise future investigations. Requirements imposed include rather detailed documentation (more involved than Category III), including a bioassay to enumerate the bioburden, a probability of contamination analysis, an inventory of the bulk constituent organics and an increased number of implementing procedures. The implementing procedures required may include trajectory biasing, clean-rooms, bioburden reduction, possible partial sterilization of the direct contact hardware and a bioshield for that hardware. Generally, the requirements and compliance are similar to Viking, with the exception of complete lander/probe sterilization.” (Ref. COSPAR Planetary Protection Policy, 20 October 2002, as amended on 24 March 2011).

Category IV specifications for selected solar system bodies are set forth in an Annex to the COSPAR Planetary Protection Policy, which also lists Solar system bodies considered to be classified as Category IV, namely, Lander Missions to Mars, Europa and Enceladus (others TBD).

PLANETARY PROTECTION CATEGORY IVB – THE EXOMARS MISSION

As the Mars lander system is carrying instruments for investigation of Martian life and does neither land nor access a Mars special regions, the ExoMars 2020 mission has been classified as **Planetary Protection Category IVb** in accordance with the ESA Planetary Protection Requirements and in agreement with the COSPAR Planetary Protection Policy.

PLANETARY PROTECTION REQUIREMENTS FOR EXOMARS

The ExoMars 2020 Planetary Protection Requirements are stated in the ESA Planetary Protection Requirements document EXM-M2-RSD-ESA-00002 issued for the project ExoMars 2020. Most of the technical Planetary Protection Requirements provided by ESA in this document can be gathered in three different groups: 1) Probability of impact, 2) Bioburden and 3) Mars samples contamination requirements.

PROBABILITY OF IMPACT REQUIREMENTS

Probability of impact requirements regarding the Launcher upper stage and the Spacecraft Composite (SCC) are the following:

- The probability of impact on Mars of the launcher upper stage shall be $\leq 1 \times 10^{-4}$ for the first 50 years after launch.
- The probability of impact on Mars by the Spacecraft composite (SCC), including CM-DM separation, shall be $\leq 1 \times 10^{-2}$.

Inputs to be included in the execution of the SCC probability of impact analysis are also provided as requirements, for example:

- Single/multiple pass analysis;
- Spacecraft reliability;
- Meteoroid impacts;
- Knowledge of spacecraft state (location, velocity vector);
- Maneuver and planet ephemeris uncertainty;
- Stochastic variability of the atmospheric density; and,
- Solar cycle.

BIOBURDEN REQUIREMENTS

Bioburden requirements deal, among other things, with the maximum number of bacterial spores allowable on the flight hardware:

- **Spacecraft:** The total bioburden of the SCC shall be $\leq 5 \times 10^5$ bacterial spores, including 20% ESA Project margin.
 - Note 1: Break-up/burn-up of the CM entering the Martian atmosphere can be used to claim bioburden reduction if the conditions of 500 C for ≥ 0.5 seconds are reached.
 - Note 2: Encapsulated bioburden is only accountable on flight hardware intended for a hard impact, e.g., CM (minus credit for break-up/burn-up), aeroshell, crushable structures.
 - Note 3: The 20% bioburden margin is intended for late contamination events and therefore would have to come out of the surface bioburden.
- **Descent Module (including the RM):** The bioburden on the Descent Module including the Rover Module shall be $\leq 3 \times 10^5$ bacterial spores on exposed internal and external surfaces and the Descent Module including the Rover Module average surface bioburden shall be ≤ 300 spores/m².
- **Rover Module (RM):** The bioburden on the RM exposed internal and external surfaces shall be $\leq 2 \times 10^4$ bacterial spores.
- **RM subsystems involved in the acquisition, delivery and analysis of Martian samples for life detection:** The average bioburden on the RM subsystems involved in the acquisition, delivery, and analysis of Martian samples for life detection shall be ≤ 0.03 bacterial spores/m².

Note that the definition of total bioburden includes surface, mated, and encapsulated bioburden.

MARS SAMPLES CONTAMINATION REQUIREMENTS

Mars samples contamination requirements refer to the maximum allowable terrestrial organic contamination level per substance class

and per gram of Martian samples for life detection. These requirements are indicated in Table 11.

Substance class	Maximum contamination level per gram of Martian sample delivered for life detection
Material from biological sources	$\leq 50 \times 10^{-9}$ gram
Monomers of Kapton, Mylar and PTFE	$\leq 500 \times 10^{-9}$ gram
Fluorinated technical lubricants	$\leq 500 \times 10^{-9}$ gram
Any other organic compound	$\leq 50 \times 10^{-9}$ gram

Table 11 – Maximum allowable contamination levels per substance class per gram of Martian sample.

OTHER PP REQUIREMENTS

There are as well more PP requirements other than the ones indicated in the three main groups above. Some examples of them are the following:

- **Induced special regions:** An analysis of flight hardware induced special regions to be provided for nominal and off-nominal mission events;
- **Organic inventory requirements:**
 - Archiving of samples of at least 50 grams of each organic material type of: (a) DM for which more than 25 kg is transported to Mars; (b) RM.
 - Documentation of SCC organic materials.
- **Methods and procedures:** is normative the use of the ECSS-Q-ST-70-53, ECSS-Q-ST-70-55, ECSS-Q-ST-70-56, ECSS-Q-ST-70-57 and ECSS-Q-ST-70-58 standards.

IMPLEMENTATION OF PLANETARY PROTECTION REQUIREMENTS FOR EXOMARS

IMPLEMENTATION OF PROBABILITY OF IMPACT REQUIREMENTS

One of the mission constraints is the probability to approach the CM-DM separation point in

off-nominal conditions (including also the failure of the separation mechanism). This could impact on the correct entry phase with consequences on the Mars contamination aspects. The occurrence probability of impact on Mars by the SCC, including CM-DM separation has to be less than 1×10^{-2} .

Probabilistic assessment is done to estimate the risk to approach Mars with an off-nominal trajectory, leading to an unwanted impact or planetary contamination. This probability is estimated with the equation:

$$P_{\text{impact on Mars}} = \sum_{i=1}^n p_i \cdot q_{i+1}$$

where:

- p_i = probability of off-nominal impact on Mars in case the i^{th} maneuver is successfully executed (and no additional correction until CM-DM separation is performed due to system failure or impossibility to exchange data and commands).
- q_{i+1} = probability to lose the system between the i^{th} and $i^{\text{th}+1}$ maneuver (this prevents any additional attitude correction and the impact conditions depend on the last (i.e., i^{th}) useful maneuver).
- The probabilities of off-nominal impact on Mars (p_i) are calculated as impact probability after each planned maneuver, as provided in the Mission Analysis.
- Probabilities q_i are estimated by combining all the causes leading to the loss of the equipment/subsystems involved in the manoeuvres: (a) H/W failures (characterized by the Reliability data), (b) Space radiation effects and (c) Micrometeoroids/Orbital debris impacts.

MICRO-METEORIDS (MM)

The MM impact analysis of the SCC is performed with relation to the MM environment described by the Grün model, as dictated by the ESA environmental specification. The effect of space debris has been ignored because they are limited to the near-Earth environment and are not present on Mars orbit environment.

The probability of failure induced by MM is assessed for all the critical hardware at unit level which is required to sustain the SCC operability during the flight phases until CM/DM separation.

For each critical component its detailed configuration was studied to identify case materials and wall thicknesses, exposed areas and the shielding effect provided by SSC structural walls, MLI blankets and adjacent SCC items. In addition, the selection of the failure mode and the most appropriate Ballistic Limit Equation (BLE) is performed. Outcome of the applied BLE are the MM critical diameters to be used to determine the critical flux, i.e., the number of MM which cause failures in this unit.

The numbers evaluated at component level are used as input to perform the SCC probability of impact analysis.

IMPLEMENTATION OF BIOBURDEN REQUIREMENTS

The first step for implementing the bioburden requirements is to understand them and then to flow down the customer PP requirements into a Project System Level PP Requirements document. Regarding the PP requirements of the DM-CM, DM-RM, Launch Vehicle-SCC and Payloads interfaces they are given in the related Interface Requirement Documents or IRDs.

The SCC bioburden budget is prepared taking into account the RM, CM and DM (without RM) bioburden budgets. Verification of the compliance of the final SCC bioburden budget with the bioburden requirements is required before the launch. Two different bioburden budgets, the Surface Bioburden Budget and the Encapsulated Bioburden Budget, are prepared for the Flight System. The Total (surface, mated and encapsulated) bioburden and the Average Surface Bioburden Density are obtained from these budgets and they have to be compliant with Planetary Protection Requirements given by the customer.

Bioburden budgets are prepared for the single modules. The final bioburden budget to be provided to the customer before the launch contains also the values of bioburden recontamination during the environmental tests, in particular the Thermal vacuum test, and the SCC recontamination during the launch campaign including the recontamination during the launch.

The CM is considered as part of the SCC with a planned hard landing because the probability of impact on Mars by the CM after DM separation is 1. The CM budget is prepared taking into account the output of the Break-up/burn-up analysis of the CM entering the Martian atmosphere. Carrier Vehicle Break-up/burn-up analysis is made considering the nominal case with inputs the state vector at the pre-separation point and a Monte Carlo simulation using the state covariance matrix at the pre-separation point. Break-up/burn-up analysis is based on a two-stage breakup model: break off of the six solar panels of the CM at a given dynamic pressure (providing a critical root bending moment) and Catastrophic breakup at which the Carrier Module with the detached solar panels totally disassembles in different parts named “debris catalogue”. The altitude at which this event occurs, the “breakup altitude” is depending on the breakup criterion selected. Three breakup criteria were considered, representing the various physical phenomena at work during the process. The most conservative break up criterion is the “Heat Load” criterion providing the lowest break up altitude and therefore the lowest heat load for each debris of the catalogue.

The analysis provides also the assessment for each debris of the thermal performance because its ultimate aim is the assessment of the self-sterilization performance of the debris (meaning a temperature at 500°C for at least 0.5s) or the bioburden reduction (the value is depending on the time above 200°C spent by the debris).

The monolithic radiative equilibrium/bulk temperature have been considered as the two thermal models providing the two “tempera-

ture extreme values”. For conservative assumption the debris temperatures from the monolithic bulk model have been selected, giving the lowest temperature. The requirement of CM total bioburden at delivery of maximum 40,000 spores is achieved by means of:

1. Break-up/burn-up (*BuBu*) analysis to identify which elements can reach the Temperature of 500°C (or higher) for a duration greater than 0.5 seconds during descent into Mars atmosphere, this condition induces sterility.
2. Active control (sterilization processing) of the elements which cannot achieve the conditions of point (1) and cannot achieve any log of bioburden reduction as result of the *BuBu* analysis.
3. Bioburden allocation for all the CM elements that cannot achieve the conditions of points (1) and (2) taking into account the log of bioburden reduction calculated with the *BuBu* analysis results.
4. Sterilization processing of the CM MLIs and CM harness by Dry Heat Microbial Reduction (DHMR) process because it is not possible to be compliant with the bioburden value allocated to the CM if the MLIs are not sterilized due to their extended exposed (internal and external) surfaces. The harness was not considered in the *BuBu* analysis due to heritage from other missions: the harness does not achieve bioburden reduction during the catastrophic break-up.

Module	Maximum Surface bioburden at delivery [spores]	Maximum Total bioburden at delivery [spores]	Maximum Surface bioburden at launch [spores]	Maximum Total bioburden at launch [spores]
SCC	#	400 000	#	500 000
CM	#	40 000	#	40 000
DM	170 000	360 000	270 000	460 000
DM w/o RM	150 000	340 000	250 000	440 000
RM	20 000	#	20 000	#

Table 12 – Bioburden allocated to each ExoMars 2020 module at the beginning of the project.

CM, RM and DM budgets are prepared at the beginning of the project in order to define the

PP strategy to be applied to each equipment and subsystem before being integrated in the module and the strategy to be applied at module and SCC level during the assembly, environmental tests, launch campaign and launch.

The general approach is to sterilize the flight hardware before integration and to reduce as much as possible the bioburden recontamination during the successive activities up to the launch. When sterilization processing is not possible due to the incompatibility of the flight hardware with any sterilization technology, cleaning and bioburden assays are mandatory up to achieve the value of bioburden allocated to the item before integration.

In general, the sequence of activities is first to clean and then to bioburden assay the item in order to know its initial bioburden level, before to apply a certified microbial reduction process. The final bioburden level of the item is then calculated considering its initial bioburden level before applying the sterilization approach and the number of log reduction obtained with the sterilization process.

When to perform the cleaning and the bioburden assays before sterilization, e.g., during and/or before the assembly, depends on the type of item and has to be established for each one before the start of the assembly.

The sterilized or cleaned items with the known bioburden level are integrated in bioburden controlled environments. Recontamination precautions and surface bioburden checks are continuously performed onto the flight hardware during the environmental tests and launch campaign in order to assure to be within the allocated bioburden levels.

Output of the bioburden budgets are the definition of the log reduction to be applied at each item with the defined sterilization process and the definition of the Assembly, Tests and Launch Operation (ATLO) environments.

Dry Heat Microbial Reduction (DHMR) as per ECSS-Q-ST-70-57C, Vapor Phase Hydrogen Peroxide bioburden reduction as per ECSS-Q-ST-70-56C, Ethylene oxide, Ultraviolet (UV) and Gamma radiation are the sterilization

methods applied for the bioburden reduction of the flight hardware and related Ground Support Equipment (GSE) and tools. All the flight hardware to be sterilized is compatible with the selected sterilization process. Indications regarding the materials and hardware compatibility tests for sterilization processes are taken from the ECSS-Q-ST-70-53 standard. Demonstration of the item compatibility with the selected sterilization process is required and it is done by analysis and/or test depending on the type of item.

Environments of different levels are used for the SCC Assembly, Tests and Launch Operations. As far as the particulate cleanroom level is concerned, cleanrooms of ISO 8, ISO 7, ISO 5 and ISO 3 level as per ECSS-Q-ST-70-01 standard are used in the project. In addition, strict bioburden controlled environments named HC (Highly Controlled) level are used during handling/operations with bioburden controlled hardware aimed to achieve or preserve the COSPAR Category IVb. Definition of these cleanrooms bioburden level is performed following the ECSS-Q-ST-70-58 and ESSB-ST-U-001 standards.

The following average surface spore density for cleanrooms in operation were used at the beginning of the project to estimate the recontamination of an item into a bioburden controlled cleanroom (The “real” bioburden recontamination numbers were then obtained as results of bioburden measurements).

- ISO class 7 cleanroom or better, highly controlled: 50 spores/m²
- ISO class 7 cleanroom or better, normally controlled: 500 spores/m²
- ISO class 8 cleanroom, highly controlled: 1,000 spores/m²
- ISO class 8 cleanroom, normally controlled: 10,000 spores/m²
- Uncontrolled manufacturing: 10⁵ spores/m²

During the handling/operations of bioburden controlled hardware, not dealing with the Mars samples, ISO 8 HC and ISO 7 HC cleanroom levels are used for the hardware integration. A

portable ISO 5 HC tent is used for transportation and introduction in a sterile environment of flight hardware which is part of the Mars sample pathway. This flight hardware area is named Ultra Clean Zone (UCZ). Sterile ISO 3 environment with controlled airborne molecular and particulate contamination is used for the ultra-cleaning and integration of UCZ flight hardware. This environment is made by a train of sterile glove boxes (GBT). GBT airborne and surface contamination are continuously controlled and monitored.

Personnel working inside the bioburden controlled environments have to be trained both in the use of sterile garments as in the behavior to be followed inside the controlled environment. Procedures for cleanroom cleaning and monitoring flight hardware cleaning and for bioburden levels maintenance during the AIT activities inside these bioburden-controlled cleanrooms are set.

A Bioburden assay plan is prepared for each item before applying the sterilization process. Bioburden assays plans are prepared at module level and at SCC level to evaluate the number of spores that can be introduced during the integration and to check, prevent and control the surface bioburden re-contamination during the flight hardware handling on ground at pre-AIT, AIT, testing and launch site operations. The bioburden assay plans are continuously adapted following the evolution of the Project.

SCC Bioburden assay plan is issued at the final phase of the mission in the last version of the Pre-launch report. The last bioburden assays to be taken at the Launch site will be included in the Post-Launch report.

Bioburden assays (Figure 44) are performed following the procedures ECSS-Q-ST-70-55C tailored for the ExoMars project by the customer.

The **bioburden assays** are performed following the bioburden assay plan issued by the ExoMars project. The plan was created taken into account the following PP requirement (given by the customer): “Bioburden assessments on

space-craft hardware shall be performed as required to meet the bioburden control plan for flight systems, but at least:

- Prior to apply a bioburden reduction procedure (e.g., DHMR);
- Prior to delivery of a bioburden controlled spacecraft hardware (and spare, if applicable);
- Prior to integration steps that inhibit further access to exposed internal and external exposed surfaces and mated surfaces – assay at last physical access – including prior to HEPA isolation;
- At the acceptance of bioburden controlled spacecraft hardware (and spare, if applicable);
- Before and after critical operations (e.g., re-work, before and after transport of major sub-systems or modules);
- After alert and action level deviations at cleanroom level;
- After any incident that could increase the bioburden for the spacecraft hardware (and spare, if applicable);
- Verification assays, prior to launch.”

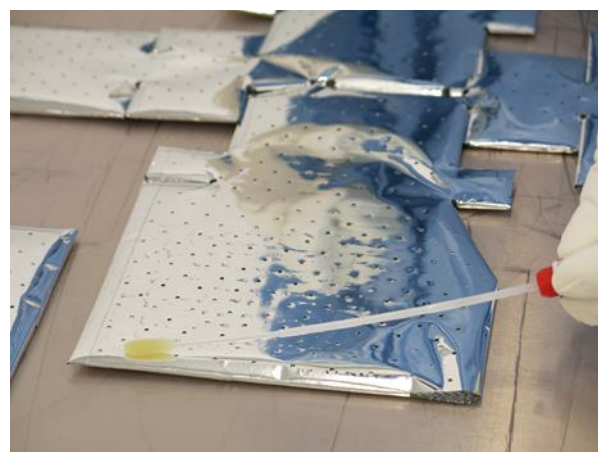


Figure 44 – Bioburden assay sampling using a swab (credit: TAS)

The number of bioburden assays to be defined for each assay set is planned taking into account the following requirements given by the customer:

- Five swabs for each surface area on spacecraft hardware of 0.1 m²;

- A proportionate number, but at least one swab, for each surface area on spacecraft hardware much smaller than 0.1 m²;
- One wipe for each surface area on spacecraft hardware in the range of 1 m²;
- Two wipes for each surface area on spacecraft hardware per 10 m².

Concerning the **encapsulated bioburden** any subsystem that undergoes a planned hard landing on Mars contributes towards the allowable encapsulated bioburden. A planned hard landing refers to all parts of the SCC subsystem that are discarded prior to the terminal descent in the nominal scenario. In the ExoMars 2020 Mission these subsystems are the CM and the DM without RM elements different from the Landing platform.

It is pointed out that planned landing profiles constitute the nominal scenario, unplanned is any off-nominal event. Hardware that undergoes an unplanned hard landing (e.g., due to landing failure) do not contribute to the allowable encapsulated bioburden.

Nevertheless, encapsulated bioburden shall be assessed for all the flight hardware, the encapsulated bioburden is allocated only for the following ExoMars 2020 SCC elements:

- DM elements that are planned to land on Mars with a hard landing (crashing onto Mars surface) as Front Shield, Rear Jacket, DM-CM separation system and Parachute system;
- CM not metallic elements that are not self-sterilized in the bulk material after the catastrophic break-up.

Estimation of the encapsulated bioburden of the flight hardware for planning purposes are performed using the following values:

- Average encapsulated spores' density of spacecraft parts not defined as electronic or not electronic parts: 130 spores/cm³;
- Electronic piece parts: 3-150 spores/cm³;
- Other non-metallic materials: 1-30 spores/cm³.

In some particular cases, e.g., Thermal Protection System (TPS), the real density of encapsulated spores (spores/cm³) can be measured with dedicated biological tests.

There is not constraint on the encapsulated bacterial spores but as the total SCC bioburden constraint is to have less than 5×10^5 bacterial spores (including the ESA margin) and the surface bioburden constraint for the DM is to have less than 3×10^5 bacterial spores (including the ESA margin), the encapsulated bioburden will have a value which will depend as the SCC materials as the surface bioburden allocation. Taking into account the total bioburden allowed to the descent module, shift surface to encapsulated bioburden allocation may be done as needed.

As these are the final values to be met at launch, a de-rating factor is applied to the H/W delivered to the Prime for the AIT with allocated values depending on their location as well as expected size, surface characteristics and AIT environments in addition to the overall maturity of the design.

The sampling and analysis of all ExoMars 2020 bioburden assays are performed by certified personnel at several Certified Microbiological laboratories (Figure 45).



Figure 45 – Microbiological Laboratory at TASInI Turin premises (credit: TAS)

Bioburden re-contamination prevention plans are prepared at modules and SCC levels in order to maintain the measured/estimated biobur-

den values of the flight hardware, recontamination prevention includes at least the following activities:

- Bioburden monitoring (of hardware and cleanroom) up to launch
- Sterile alcohol wiping
- Sterile covers/biobarriers/packaging material
- Sterile fluids (e.g., N₂) in contact with the flight hardware
- Use of HEPA filters, bioseal
- Bioburden control of Ground Support Equipment (GSE) in contact with the flight hardware or entering in controlled CRs
- Contingency planning
- Definition and use of cleaned transport containers with dedicated filters
- Training Program for all personnel involved with controlled environments

Recontamination prevention systems as temporary biobarriers, covers, overpressure flow systems, etc. are implemented during ATLO activities starting with the SCC design definition (Figure 46).



Figure 46 – Temporary biobarriers covering flight hardware placed into a bioburden controlled cleanroom at TASinI premises (credit TAS)

As far as the bioburden requirement of the RM subsystems involved in the acquisition, delivery, and analysis of Martian samples for life detection is concerned, the required average bioburden requirement of having less than 0.03 bacterial spores/m² is achieved by implementation of a dedicated strategy. The first step was to identify the RM surfaces that will be directly in contact with the Mars sample and the

RM surfaces which enclose the volume (environment) that will be in contact with the Mars sample without being directly in contact with the sample (all the RM surfaces making part of the Martian sample pathway since the Mars sample extraction from the Mars subsurface up to final analysis by the analytical instrumentation). This zone is called “Ultra-Cleaned Zone” or UCZ.

The PP strategy consists of disassembling the flight hardware in order to have all the UCZ parts available for cleaning and sterilization. The cleaning is made with several solvents of different polarity including sterile isopropyl alcohol 70% in order to remove the bacterial spores other than the organic contaminants. High performance techniques and related procedures of cleaning and packaging are developed. The cleaned UCZ parts are sterilized by Dry Heat Microbial Reduction (DHMR) process in order to achieve the required average surface spore density less than 0.03 bacterial spores/m². The transportation of the packed sterilized parts into a sterile environment is made without breaking the sterilization chain through a dedicated portable tent ISO 5 HC level. Dedicated procedures for handling, transportation into the portable tent and introduction of the items into the sterile environment are developed. These activities are performed by personnel wearing sterile garments as bunny suits, boots, double gloves and mask.

The unpacking, ultraclean with CO₂ snow cleaning process, integration and test of the sterile disassembled UCZ parts is made in an isolate sterile environment. This environment is built as a “train” of sterile glove boxes, the Glove Box Train or GBT. The GBT is sterilized with Vapor Phase Hydrogen Peroxide technique. The GBT has an extremely controlled atmosphere with respect to particulate, molecular and biological contamination. Regarding the biological contamination, the airborne and surface biological level of each glove box is monitored by the Microbiological laboratory by means of bioburden air sampling and fall out plates placed in each glove box. Dedicated procedures and monitoring plan are

developed. The bioburden levels required for each glove box are 0 CFU per m³ of air and 0 CFU per fall-out plate in order to assure no bioburden recontamination occurs during the UCZ integration inside the GBT (Figure 47).



Figure 47 – ISO 5 movable tent and sterile GBT at TASin/Turin premises (credit: TAS)

Once the UCZ is integrated is over-pressurized on ground with sterile gas and maintained until landing on Mars to prevent the bioburden (and organic) recontamination.

Analytical Payloads parts that belong to UCZ are also treated by the payloads providers in order to achieve the required average surface spore density less than 0.03 bacterial spores/m². Sterilization process is applied on the parts. Dedicated strategies for packaging, transport, unpack and integrate the related analytical payloads parts with the other UCZ components are developed and implemented.

IMPLEMENTATION OF MARS SAMPLES CONTAMINATION REQUIREMENTS

The first step to approach the requirements is to understand them and to define which spacecraft materials could directly or indirectly contaminate the Martian sample since it is extracted with the drill tool till is analyzed with the RM analytical payloads. A bottom-up approach is applied to derive the contamination of the Martian sample at the End of life sample as a sum of different contributions to the sample contamination. The following contributions to the Martian sample contamination are evaluated:

- UCZ surface contamination (residue of ultra-cleaning, molecular and particulate contamination during UCZ AIT);

- UCZ airborne contamination (off-gassing of UCZ materials);
- Off-gassing of RM external surfaces (including contamination from plumes, Entry Descent Landing re-pressurization);
- Drill tool chamber contamination (off-gassing drill materials, molecular and particulate contamination during Drill AIT, contamination from plumes, Entry Descent Landing re-pressurization).

The UCZ molecular organic contamination (MOC) and particulate contamination (PAC) are evaluated through witness samples placed inside the GBT. Static contact transfer coefficients for the UCZ static parts and Dynamic transfer coefficients for the UCZ not static parts are used to evaluate the transfer of UCZ surface contamination to the Martian sample.

The UCZ airborne molecular contamination is evaluated considering the outgassing of contaminants from polymeric materials present in the UCZ. At the beginning of operations on Mars the core sample transport mechanism door is opened and the UCZ pressure is equalized to the Martian atmosphere pressure. Therefore, the outflow of contaminants occurs and the UCZ airborne molecular contamination is reduced. The contribution to the molecular contamination of the Martian sample due to the adsorption of molecular contamination coming from the UCZ atmosphere is evaluated taking into account sticking factors and the reduced UCZ airborne molecular contamination.

Molecular contamination coming from the thrusters Plume impingement and exhaust gases can contaminate the external surfaces of the RM. In addition, off-gassing of the not metallic materials present on the external surfaces of the RM can occur in the Mars atmosphere. Once extracted from the Mars subsurface the sample temperature can influence the transport of contaminant if it is colder than the environment, facilitating the condensation of molecular contaminants which can be transported from the external surfaces of the RM to the exposed sample. Evaluation of this contribution to the Martian sample contamination is performed by means of CFD analysis taking into account convection-diffusion transport in the

flow regime present on Mars, the molecular contaminants off-gassing from the non-metallic surfaces of the RM and the temperature distribution on the RM as the surrounding environment.

Molecular contamination coming from the thrusters Plume impingement and exhaust gases as well as particulate contamination dislodging from the surfaces of descent module can inflow into and contaminate the UCZ parts of the drill tool. This contribution is also considered to evaluate the Mars sample contamination due to the drill UCZ parts in addition to the UCZ contamination acquired during the AIT and UCZ airborne contamination due to UCZ materials off-gassing. Adsorption and dynamic transfer coefficients are used for the evaluation.

A Martian sample contamination budget is defined allocating contamination amounts to each mission phase. Outputs of this budget are the definition of the UCZ cleaning processes and the definition of the required UCZ AIT controlled environments (maximum acceptable particulate and molecular contamination levels of the GBT air and surfaces) based on the maximum allowed UCZ surfaces contamination. GBT molecular and particulate monitoring procedures and plans are issued together with the definition and implementation of monitoring procedures to check the molecular and particulate levels of the UCZ surfaces during the AIT made inside the GBT. Reviews of the flight hardware materials are performed taking into account the different contributions to the Martian sample contamination.

Over-pressurization of the UCZ prevents the organic, particulate and biological UCZ recontamination.

Several “blanks” are tested to prove that the integrated Mars sample pathway contain “no biological” and “no organic” terrestrial contamination which could impact the test results when searching the life in Mars.

PLANETARY PROTECTION REQUIREMENTS VERIFICATION

The verification of the PP requirements is progressively controlled by the issue of Verification Control Documents (VCDs) that are continuously adapted up to the end of the program. For each PP requirement are indicated the verification methods at the applicable level. The verification methods might be satisfied by Test, Analysis, Inspection, Review of Design or a combination of multiple of verification techniques. In general:

1. Probability of impact requirements are verified by analysis;
2. Bioburden requirements are verified by test and analysis;
3. Mars samples contamination requirements are verified by test and analysis.

LAUNCH CAMPAIGN RECONTAMINATION ON GROUND AND AT LAUNCH

During the launch activities on ground the surface bioburden, particulate and organic recontamination has to be monitored including the period during which the SCC is encapsulated into the Payload Fairing (PLF) up to launch. The recontamination values obtained during the bioburden particulate and organic monitoring are included in the respective contamination budgets.

After the SCC encapsulation into the PLF the PLF is connected to the Thermal Control Units of the train and Launch Pad. Evolution of the bioburden contamination into both the PLF and the DM is computed by analysis in order to evaluate the recontamination of the DM internal exposed surfaces.

The bioburden recontamination due to the acoustic environment at launch is evaluated making a direct proportion between the area of the rear jacket valves and the area of the hatches of the fairing. The recontamination due to transfer effects from the fairing to the Rear Jacket during the launch is estimated by dedicated analysis.

All the obtained recontamination values are included in the final SCC bioburden budget.

PLANETARY PROTECTION DOCUMENTATION

The required Planetary Protection documentation with indication of the related reviews is listed in Table 13. The procedures of the related reviews contain the complete list of the Planetary Protection documents to be delivered in the respective review. This list can be updated following the program evolution.

THINGS TO REMEMBER

- Fundamental to provide PP courses for all project team at earliest phase of the project specially to the subsystem engineers, before the selection of the subcontractors and before the definition of the flight H/W materials and design.
- Take into account the lessons learned coming from previous projects with similar PP category.

- Ensure to place the Prime PP requirement specification at the same level of the system specification as normative document to flow down the PP requirements to the low level specifications.
- Made an accurate evaluation of the PP Implementation costs since the beginning of the project.
- To include time-demanding activities as hardware cleaning, microbial assays and sterilization process in the AIT schedule to avoid several criticalities in the AIT and Launch Operations plans.
- As PP is a transversal competence is needed to ensure a good communication and effective relationships between PP team and project manager, engineers, ATLO personnel, laboratories personnel and subcontractors.

Documentation	Draft	Final	PPO Approval/Review
Planetary Protection Implementation	-	SRR	A
Requirements			
Planetary Protection Plan	SRR	PDR	A
Planetary Protection Implementation Plan	PDR	CDR	A
Pre-Launch Planetary Protection Report	FAR	FRR	R
Post-Launch Planetary Protection Report		As required	R
End-of-Mission Planetary Protection Report		As required	R
Organic Material Inventory	CDR	FRR	R

Table 13 – Planetary Protection documentation.

PAYLOAD CASE STUDY²⁷

This section describes the planetary protection activities undertaken prior and during the assembly and test of the DREAMS instrument on board of ExoMars Entry Descent Module (EDM) launched in 2016.

PAYLOAD OBJECTIVES

DREAMS is the autonomous surface payload package accommodated on the ExoMars EDM launched in 2016. It is a meteorological station with the additional capability to perform measurements of the electric fields close to the surface of Mars.

DREAMS scientific objectives are:

- Meteorological measurements by monitoring pressure, temperature, wind speed and direction, humidity and dust opacity during a Martian sol (10 measurements during daytime and 2 at night) at the landing site.
- Characterization of the Martian boundary layer in dusty conditions.
- Hazard monitoring by providing a comprehensive dataset to help engineers to quantify hazards for equipment and human crew: velocity of windblown dust, electrostatic charging, existence of discharges, and electromagnetic (EM) noise potentially affecting communications.
- The first ever investigation of atmospheric electric phenomena at Mars.

The DREAMS package included the following subsystems:

- DREAMS-H (humidity sensor)
- DREAMS-P (pressure sensor)
- MarsTem (thermometer)
- MetWind (wind sensor)
- MicroARES (electric probe)
- SIS (Solar Irradiance Spectrometer)
- CEU (Central Electronic Unit)
- Battery

PLANETARY PROTECTION REQUIREMENTS

Being part of a Category IVB mission, DREAMS was subject to PP requirements from the top level of the mission.

DREAMS subunits were assembled in ISO 7 cleanroom environment. Common contaminants found in cleanroom were generated from four basic sources: the facility, people, tools, and product generated. Areas adjacent to the cleanroom were less-clean than the cleanroom; material airlock and clothing-change area were contaminated by the activities going on in these areas, and the contaminations in the outside corridors and service plenums were not be controlled. The air supplied to the cleanroom, if not correctly filtered, was a source of contamination. The floor, walls, ceiling and other surfaces in the cleanroom were surface sources, their contamination being mostly derived from personnel touching them, their contamination depositing from air. These surfaces were also sources of contamination if poor quality constructional components were used, which break up and disperse fibers, wood chips, plaster, etc. Cleanroom clothing, gloves and masks are other surfaces that are contaminated either by the people wearing them or by other cleanroom surfaces. Personnel within the cleanroom can disperse contamination from the skin, mouth and clothing. This contamination can be transferred to the sample through the air, or by contact with their hands or clothing. Machines are another source, as they can generate contamination by the movement of their constituent parts, or by generation by thermal, electrical or other means. Raw materials, sample containers and packaging that were brought in, or piped into the DREAMS cleanroom, could have been contaminated and were considered as sources.

BIOBURDEN ESTIMATION

The best estimate of bio-burden on DREAMS were based on estimated free and enclosed surface areas, mated surfaces, and encapsulated

²⁷ This section has been contributed by John Brucato (Dreams payload and MAMIS).

non-metallic volumes of structures and components. Estimates of bio-burden levels assumed that all hardware undergoes microbial reduction procedures. Procedures for preparing standard swab assay will follow those reported in ECSS-Q-ST-70-55C. According to ExoMars top levels requirements, the payload shall have a cleanliness level of 300 spores/m² for external general surfaces (i.e., defined as non-sensitive) at delivery for integration onto the EDM.

Determining microbial burden allocations for the hardware required detailed dimensions and specifications of all components and subsystems making up DREAMS. Enclosed surfaces, exposed surfaces, enclosed volumes and encapsulated volumes were determined for each component. Mated surfaces are not tallied and a conservative assumption of the total mated area of DREAMS hardware was assumed, and accounted for as free surface spores.

Surface bioburden depends on the class of the clean room in which the subsystem is assembled.

MEETING THE PP REQUIREMENTS

The determination of colony forming units (cfu) after plating of cultivable microorganisms from surfaces and from the air was made according to ESA ExoMars tailored bioburden assay procedures from ECSS-Q-ST-70-55C. All air samples were analyzed according to the ESA Standard air sampling (no heat shock). The cleanroom temperature was 24 ± 2 °C and a positive pressure differential was maintained between the cleanroom and the outside. The relative humidity was $45 \pm 10\%$.

For each surface, the number of samples to be taken was calculated as:

$$\#samples = \sqrt{[A_{tot}(cm^2)/25(cm^2)]}$$

Swab assay

A sufficient number of sterile dry swabs (Copan FLOQSwab, 552C), 2ml Eppendorf tubes, sterile 15ml Falcon tubes, and 500ml glass bottles were prepared. We autoclaved the Eppendorf tubes, and the water in the 500ml glass

bottles. Aseptically (i.e., in laminar flow cabinet) we pipetted 1ml of sterile water into each sterile Eppendorf tube and 2.5ml of sterile water into each sterile Falcon tube.

In the cleanroom sterile swab was removed from its container and moisten the head of the swab using the sterile water in the sterile Eppendorf tube. Express excess moisture from the swab against the interior wall of the tube.

The swab was hold so that the handle makes about a 30° angle with the surface to be sampled. While moving the swab in one direction, we rotated the head of the swab slowly and thoroughly over a measured 25 cm² surface area. The linear direction of the swabbing motion 90° was changed and again the surface swabbed thoroughly. We completed a third coverage of the surface by again changing the direction of the swabbing motion by 135°.

The swab was putted in a sterile Falcon tube containing 2.5ml sterile water, we brake the swab shaft at the breakpoint, and closed the Falcon tube for further processing.

Transport and storage

Samples were transported to the laboratory, stored at 4-8°C and processed within 24 hours.

Extraction

Each Falcon tube containing the water and the swab was placed on a vortex mixer and vortex at maximum power for 5-6 seconds.

Each Falcon tube containing the water and the swab was then placed in an ultrasonic bath making sure the liquid level in the bath is above the fluid level in the tubes and sonicated for 120 ± 5 seconds with a power of 240W and a frequency of 35kHz.

Heat shock

The Falcon tube containing the vortexed and sonicated swab was placed in a water bath at 80 ± 2 °C for 15 minutes, as determined by a pilot tube containing a thermometer. Make sure the liquid level in the water bath is above the fluid level in the tube.

After heat shock, we cooled the Falcon tube rapidly to bring the contents to 30-35°C. The

entire plating procedure requires more than 10 minutes, thus the heat shocked tube shall be placed in an ice bath for no longer than 45 minutes prior to plating.

Plating

Each Falcon tube containing the water and the swab was placed on a vortex mixer and vortex at maximum power for 2 seconds. Aseptically (i.e., in laminar flow cabinet) we pipetted 0.5ml aliquots onto the surface of 4 R2A Petri plates.

We used a sterile spreader to spread the dilution over the surface as evenly as possible. Allow the moisture to be absorbed into the agar before incubation.

Incubation

Plates were incubated at $32 \pm 1^\circ\text{C}$.

Counting

We examined the plates at 24 and 48 hours. If colonies were visible by eyes, we counted and recorded data. We examined and recorded final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72-hour count is made.

Controls

For each ten or fewer samples collected, we also collected a 'field negative' control, at least 3 per day. In the cleanroom we removed sterile swab from its container and moisten the head of the swab using the sterile water in the sterile Eppendorf tube. Express excess moisture from the swab against the interior wall of the tube.

We waved the moistened swab through the air for 2 to 4 seconds and putted the swab in a sterile Falcon tube containing 2.5ml sterile water, broken the swab shaft at the breakpoint, and closed the Falcon tube for further processing.

In the lab, we created at least two 'lab negative controls'. In the laminar flow cabinet, we removed sterile swab from its container and moistened the head of the swab using the sterile water in the sterile Eppendorf tube. Express excess moisture from the swab against the interior wall of the tube.

We putted the swab in a sterile Falcon tube containing 2.5ml sterile water, broken the swab shaft at the breakpoint, and close the Falcon tube for further processing.

We analyzed the controls in the same way as the samples described above.

Some examples of corresponding areas of DREAMS (in blue) sampled are reported in Figure 48 and Figure 49.

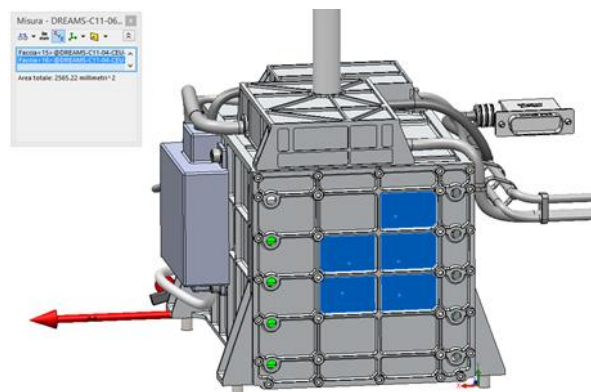


Figure 48 – Area in blue represents an example of how to report places where swab sampling was executed.

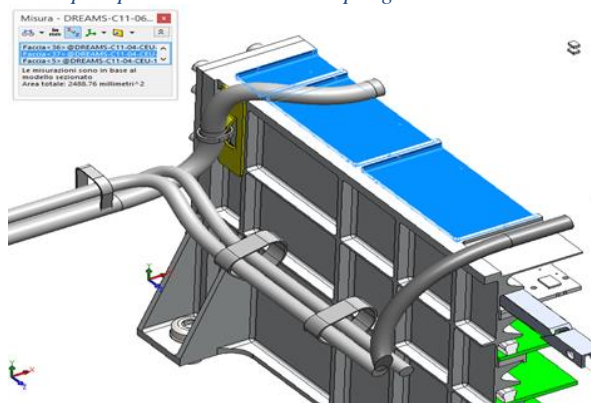


Figure 49 – Area in blue represents an example of how to report places where swab sampling was executed.

VERIFYING THAT THE REQUIREMENTS ARE MET

The Dry Heat Microbial Reduction was performed on DREAMS in the thermo-vacuum chamber equipped with twelve Pt100 temperature sensors to monitor the effective temperature at different chamber locations. Temperature was maintained at 115°C for 9 hours.

After thermo-vacuum tests, DREAMS were swab sampled to control the final bioload. Total counting of zero spores were measured and according to zero total case, a total bioload of

53 cfu were measured on DREAMS flight model.

DREAMS after bioburden reduction, showed average bioburden density less than 300 bacterial spores/m² on exposed surfaces. Having measured the bioload to be well below the Planetary Protection Implementation Requirement levels, the flight hardware was delivered

to TAS-F under supervision of ASI in double barriers to preserve its sterility and bioburden allocation during shipping. Reports were prepared and approved by the Planetary Protection Manager and authorized by the PPO.

CASE STUDY FOR PLANETARY PROTECTION CATEGORY V – UNRESTRICTED EARTH RETURN: HAYABUSA-1 & 2²⁸

The main characteristics of the Hayabusa-1 and 2 missions are described in Table 14.

The Hayabusa-2 mission successfully reached its target asteroid Ryugu, arriving at 20 km altitude from the asteroid surface on June 27, 2018 (Figure 50). First rover deployment occurred in September and October and sampling will occur in early 2019, before the probe starts its one-year long journey back to Earth in December 2019.

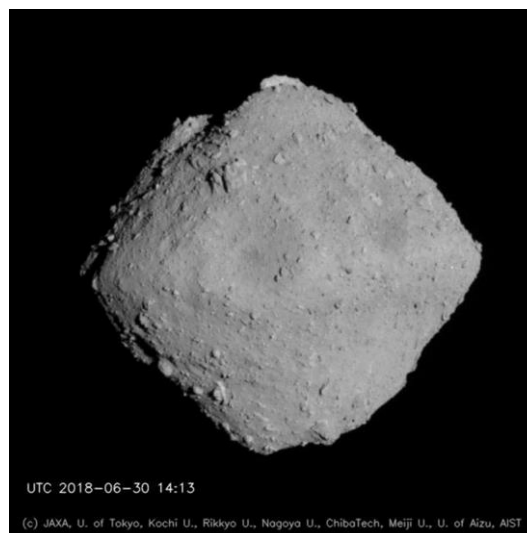


Figure 50 – Asteroid Ryugu as seen by Hayabusa-2 on 30 June 2018 (Credit: JAXA).

	Hayabusa-1	Hyabusa-2
Objectives		
Mission Target	Itokawa: S-type , sub-km NEO	Ryugu: C-type , 1-km NEO
Major Payload Instruments	<ul style="list-style-type: none"> •Surface Sampling System x3 •Earth Return Capsule •Multi-band Optical Camera •LIDAR •Near Infrared Spectrometer •X-ray Fluorescence Spectrometer •Micro-Rover x1 •Target Markers x3 	<ul style="list-style-type: none"> •Surface and Sub-surface Sampling System x3 •Earth Return Capsule •Small Carry-on Impactor with DCAM •Multi-band Optical Camera •LIDAR •3 μm Near Infrared Spectrometer •Thermal Imaging Camera •Micro-Rovers x3 •Micro-Lander x1 •Target Markers x3
Mission Epoch	2003-2010	2014-2020

Table 14 – Main characteristics of the Hayabusa-1 and 2 missions.

PLANETARY PROTECTION CATEGORY V DESCRIPTION

The COSPAR Planetary Protection Policy²⁹ (Table 15) describes Category V as follows:

“**Category V missions comprise all Earth-return missions.** The concern for these missions is the protection of the terrestrial system, the Earth and the Moon. (The Moon must be protected from back contamination to retain

freedom from planetary protection requirements on Earth-Moon travel).

For solar system bodies deemed by scientific opinion to have no indigenous life forms, a subcategory “unrestricted Earth return” is defined. Missions in this subcategory have planetary protection requirements on the outbound phase only, corresponding to the category of that phase (typically Category I or II).

²⁸ This section is based on a presentation by Dr. Hajime Yano, with Toshi Chujo, JAXA/ISAS, Japan and the Hayabusa-1 and Hayabusa-2 Teams.

²⁹ Published in *Space Research Today*, COSPAR's information bulletin, Number 200, December 2017 after having been approved by the COSPAR Bureau at its meeting in March 2017.

For all other Category V missions, **in a subcategory defined as “restricted Earth return”, the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware** which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth.

Post-mission, there is a need to conduct timely analyses of any unsterilized sample collected and returned to Earth, under strict containment, and using the most sensitive techniques. **If any**

sign of the existence of a non-terrestrial replicating entity is found, the returned sample must remain contained unless treated by an effective sterilizing procedure.

Category V concerns are reflected in requirements that encompass those of **Category IV plus a continuing monitoring of project activities, studies and research** (i.e., in sterilization procedures and containment techniques).”

All sample return missions to the Earth-Moon system should be sub-divided into restricted and unrestricted Earth return, depending upon the confidence degree of terrestrial biosphere contaminations by these samples.

Category I (Forward)	Flyby, Orbiter, Lander	Differentiated, metamorphosed asteroids, Io, others TBD
Category II (Forward)	Flyby, Orbiter, Lander	Venus, Moon (organic inventory), Comets, Carbonaceous Chondrite Asteroids, Jupiter, Saturn, Uranus, Neptune, Ganymede, Callisto, Titan, Triton, Pluto/Charon, Ceres, Kuiper Belt Objects, others TBD
Category III (Forward)	Flyby, Orbiter	Mars, Europa, Enceladus, others TBD
Category IV (Forward)	Lander	Mars, Europa, Enceladus, others TBD
Category V (Backward)	Sample Return	(a) Restricted: Mars, Europa, Enceladus, others TBD
		(b) Unrestricted: Venus, Moon, P/Wild2, Itokawa, Bennu, Ryugu, others TBD

Table 15 – COSPAR Planetary Protection Policy: Restricted Earth return vs. Unrestricted Earth return (Kminek et al., 2017).

REQUIREMENTS FOR CASE STUDY

According to 1998 report of the US National Research Council’s Space Studies Board entitled “Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies: Framework for Decision Making” (SSB, 1998), parameters relevant to assess the potential for presence of a biological entity in returned samples should be considered as follows:

- **Liquid water:** Liquid water may safely be considered a requirement for life on small solar system bodies, because the chemistry on which life is based must take place in solution, and there is no other plausible solvent.
- **Energy sources:** A source of energy to support the origin and continuation of life

in any environment is a thermodynamic necessity. For the extraterrestrial environment, the energy sources are both geochemical and photosynthetic.

- **Organic compound:** Chemical building blocks for organic polymers must be available.
- **Temperature:** The temperature limits for the survival of metabolically active cells (160 °C) at 1 atm are likely to apply to extraterrestrial organisms also unless their biochemistry does not depend on the formation of amide, ester or phosphodiester bonds.
- **Radiation intensity:** Extraterrestrial biopolymers are unlikely to differ greatly from terrestrial biopolymers with respect to radiation sensitivity.

- **Comparison to natural influx to Earth:** Earth receives natural influx of extraterrestrial material, mainly in the form of cosmic dust. Some materials may be delivered in ways that shield it from sterilizing temperatures or radiation.

CATEGORY V DESCRIPTION IN THE CASE OF SAMPLE RETURN MISSIONS FROM SMALL SOLAR SYSTEM BODIES

Determination as to whether a mission is classified “Restricted Earth return” or not shall be undertaken with respect to the best multidisciplinary scientific advice, using the framework presented in (SSB, 1998).

Specifically, such a determination shall address the following six questions for each body intended to be sampled:

1. Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?
2. Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?
3. Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO₂ or carbonates and an appropriate source of reducing equivalents) in or on the target body to support life?
4. Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., > 160°C)?
5. Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life forms?
6. Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

For containment procedures to be necessary (“Restricted Earth return”), an answer “no” or

“uncertain” needs to be returned to all six questions.

For missions determined to be Category V, “Restricted Earth return,” the following requirements shall be met:

- Unless specifically exempted, the outbound leg of the mission shall meet contamination control requirements to avoid “false positive” indications in a life-detection and hazard-determination protocol, or in any search for life in the sample after it is returned. A “false positive” could prevent distribution of the sample from containment and could lead to unnecessary increased rigor in the requirements for all later missions to that body.
- Unless the samples to be returned are subjected to an accepted and approved sterilization process, the canister(s) holding the samples shall be closed, with an appropriate verification process, and the samples shall remain contained during all mission phases through transport to a receiving facility where it (they) can be opened under containment.
- The mission and the spacecraft design must provide a method to “break the chain of contact” with the small body. No uncontained hardware that contacted the body, directly or indirectly, shall be returned to Earth. Isolation of such hardware from the body’s environment shall be provided during sample container loading into the containment system, launch from the body, and any in-flight transfer operations required by the mission.
- Reviews and approval of the continuation of the flight mission shall be required at three stages: 1) prior to launch from Earth; 2) prior to leaving the body or its environment for return to Earth; and 3) prior to commitment to Earth re-entry.

For unsterilized samples returned to Earth, a program of life detection and biohazard testing, or a proven sterilization process, shall be undertaken as an absolute precondition for the controlled distribution of any portion of the sample (SSB, 1998).

I No special containment and handling warranted beyond what is needed for scientific purposes		II Strict containment and Handling warranted
Ia <i>High Degree of Confidence</i>	Ib¹ <i>Lesser Degree of confidence</i>	
Moon Io Dynamically new comets ² Interplanetary Dust particles ³	Phobos Deimos Callisto C-type asteroids (Ryugu) Undifferentiated metamorphosed Asteroids (Itokawa, Bennu) Differentiated asteroids <i>All other comets (P/CG)</i> Interplanetary dust particles ³	Europa Ganymede Enceladus P-type asteroid ⁴ <i>D-type asteroids⁴</i> (Jupiter Trojans) Interplanetary dust Particles ³

¹ Evaluation on case by case basis.

² Samples from the outer 10 meter.

³ Samples from the same parent bodies of this group.

⁴ Limitation of available data led to a conservative assessment => need for containment

Table 16 – Amended by COSPAR (March 2005) adapted from the Space Studies Board, National Research Council, Evaluating the biological potential in samples returned from Planetary Satellites and small Solar System Bodies, Task Group on Sample Return from small Solar System Bodies, National Academy of Science, Washington, D.C., 1998.

Table 16 summarizes the Category V provisions for small solar system bodies.

Answers to the six questions mentioned above will now be reviewed in the case of Hayabusa.

Question #1: Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?

Answer #1: “UNCERTAIN”. Recent discoveries provide mounting evidences that there was water on and in main belt C-type asteroids. However, “liquid” cases for NEOs of the same type are less certain.

- Aqueous alteration of primitive parent body material is well known from carbonaceous chondrite samples, in which liquid water penetration likely ended billions of years ago.
- Water ice and organic absorption signatures are recently reported for the surface of (24) Themis (C-type main belt asteroid).
- Discovery of “main belt comets” raises a possible water presence inside Co-location of hydrated minerals and organic compounds are suggested for carbonaceous chondrites.

- Dormancy period of hypothetical spores must be in the order of billions of years in a dry environment.

Question #2: Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?

Answer #2: “NO”. Primitive meteoritic material could provide sufficient energy resources to potential life-forms.

- Both photosynthetic and chemical processes are considered at the near earth space.
- Chemical reduction-oxidation (red-ox) reactions playing the key role
- Mineralic components like phyllosilicates, sulfides, phosphates, carbonates, silicates provide nutrients like S, P, Ca, Mg, K, Fe, Cl, etc.
- Mautner *et al.* (1997) reported that microbial life and plants have been grown on Murchison extracts.

Question #3: Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO₂ or carbonates and an appropriate source of reducing equivalents) in or on the target body to support life?

Answer #3: “NO”. Carbonaceous meteorites contain organic material to support growth of organisms.

- Primitive meteoritic material usually contains a “few” percent of carbon
- Carbon phases are ubiquitous in primitive meteorites
- Tagish Lake meteorites have up to 5% organic content (D/T-type?)
- Callahan *et al.* of NASA/GSFC (2011) reported that some nucleobases such as DNA blocks (Adenine, Guanine) and nucleobases analogs were extracted from Antarctic meteorites
- Organic inventory makes them scientifically interesting

Question #4: Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., > 160° C)?

Answer #4: “NO” for most of pristine materials as 1999 JU3 perihelion does not exceed the recommended temperature on its surface. “YES” for local heat maximums like impact craters.

- Usually in meteorites there is no evidence that this temperature has been exceeded significantly.
- Surface temperatures usually do not exceed 130°C in NEO orbits, unless very close perihelion (e.g., 1989 UQ @ 0.67 AU > 200° C).
- Impact processes create very local extreme temperatures.

Question #5: Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life-forms?

Answer #5: “YES”. Given the extremely long exposure time with slow turnover rate, a sterilization of the top surface is assumed. Also sub-surface materials for both monolithic bedrock and regolith layers to be excavated by artificial cratering are assumed to be sterilized by

galactic cosmic rays and radionuclides contained in the carbonaceous chondritic interior of the target body.

This question deserves more discussion in the case of Hayabusa-2, which involves two sampling sequences:

- (1) Surface Sampling, the same as the Hayabusa-1
 - Safe area (no boulders at s/c size in the landing ellipse)
 - Choose the sites for both scientific and operational merits
 - Up to two different locations
 - Impact sampling by projectiles

In this case, sterilization is sufficient by solar UV radiation, vacuum, and other space parameters, as illustrated in Figure 51.

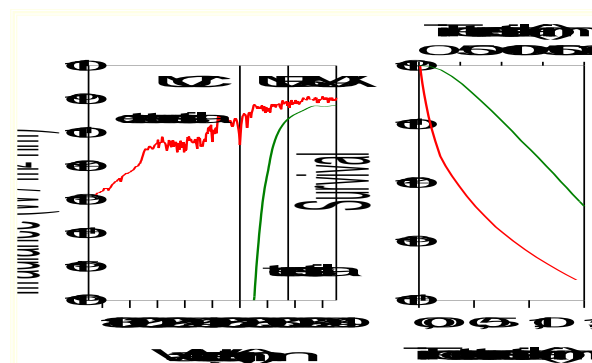


Figure 51 – Tens of seconds of exposure to extraterrestrial solar UV radiation in space killed 99% of bacterial spores (Experiment on Spacelab 1: *B. subtilis* spores) (After Horneck G. et al., MMBR, 2010).

- (2) Sub-surface sampling to be attempted a few weeks after SCI crater formation
 - If the new fresh crater is identified
 - If temporal dust torus settled down to be safe
 - If the vicinity of the artificial crater meets safety requirement of landing
 - Only once, either inside or just outside of the crater
 - Total mass is at a range of > 100 mg

In this case, sterilization process must be examined as a function of time and depth.

The deepest depth estimate of the artificial crater on regolith layers is ~200 cm and sampling attempt will be taken place a few weeks

after the crater formation (UV sterilization is in effect to some degree).

Sub-surface sterilization processes assessment results in the following conclusions:

- (1) Sub-surface Temperature level
 - Heat sterilization of indigenous life in 200 cm depth sub-surface of 1999 JU3 does not seem likely.
- (2) Penetration of GCR, with gardening effect
 - Gardening depth and time in the local area are unknown; yet Itokawa proved granular convection on the sub-km asteroid and its samples experienced < 8 Myr GCR exposure. The ~200 cm-deep crater floor of 1999 JU3 regolith layers may be at the marginal depth of shield limit to protect indigenous life in < 1.1 Myr (Mileikowsky *et al.*, 2000) or in > 10 Myr (Clark *et al.*, 1999) with an uncertainty.
- (3) Radionuclides exposure inside carbonaceous chondrite composition
 - Carbonaceous chondrites contain a number of long-lived radionuclides and its aqueous alteration occurred inside the parent body of 1999 JU3 right after its accretion at ~4.5-3.5 Ga. It is long enough to provide sufficient dosage to kill indigenous life by the present time.

Question #6: Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth e.g., via meteorites, of material equivalent to a sample returned from the target body?

Answer #6: “YES”. With variations over time, the asteroidal (and certainly NEO) material including carbonaceous chondrites and micro-meteorites has been collected on the Earth in large quantities.

- Spectral comparison to date considers carbonaceous chondrites as representative of C-type asteroids.

- There are indications that certain materials are underrepresented in the world’s meteorite collection (e.g., brittle carbonaceous material).
- Today’s incoming stream of meteoritic material may be not representative and may vary over time.
- For NEO, the material should have been already arrived at the Earth.

IMPLEMENTATION OF REQUIREMENTS FOR CASE STUDY

The Hayabusa-2 project was officially approved for the Japanese Fiscal Year 2011 (FY-2011). In January 2012, the Space Activities Commission of the Government of Japan approved the Hayabusa-2 mission. Manufacturing of subsystems started in FY-2012. In May 2012, the NAC Planetary Protection Subcommittee gave a presentation of Hayabusa-2. That same month, the COSPAR PPP Colloquium at Alpbach recommended the categorization. In July 2012 at the COSPAR Scientific Assembly in Mysore (India), a PPP resolution was granted.

From January to April 2013, the first interface tests took place. From October 2013 to September 2014, FM integration tests were carried out. In July 2014, at COSPAR Scientific Assembly in Moscow (Russia), Mars impact probability was reported and accepted. In December 2014, the spacecraft was launched by H-IIA from the Tanegashima Space Center.

In December 2015, Hayabusa-2 was subject to Earth gravity assist. Arrival to the target asteroid Ryugu occurred in June 2018. In October 2018, the first rover deployment and the first sampling were made. Departure from Ryugu will be in December 2019. In December 2020, Hayabusa-2 will return to Earth and land at Woomera, Australia with asteroid samples.

Implementation of requirements pertaining to orbital analysis for impact probability on Mars has been dealt with in the section about Orbital Dynamics and Impact Probability Analysis.

THINGS TO REMEMBER

- All sample return missions regardless of the target body are in Category V.

- In the Category V, there are two sub-categories as restricted and unrestricted earth returns (RER & UER).
- RER targets such as Mars, Europa, and Enceladus must be scientifically prepared for protections of both (potential) biospheres in outbound and inbound legs.
- The Category V missions to solar system small bodies are determined their categorizations on a case-by-case basis, based upon the best scientific knowledge at the time of the category proposal to COSPAR PPP.
- In order to judge RER/UER categorizations, small body sample return missions must go through the six question series.
- If the Category V UER category is granted by COSPAR, no further range of requirements are requested, except regular mission progress reports for all and Mars impact probability analysis for those missions having a potential to go beyond the Martian orbit.
- Mission design and spacecraft system may be affected by the PPP categorization. Act early to propose the COSPAR PPP categorization and get approved prior to finalizing the mission scenario and design.

Mautner, M.N., Conner, A.J., Killham, and K., Deamer, D.W. (1997). Biological Potential of Extraterrestrial Materials. *Icarus*, 129, 1, 245-253.

Mileikowsky, C., Cucinotta, F.A., Wilson, J.W., Gladman, B. Horneck, G., Lindegren, L., Melosh, J., Rickman, H., Valtonen, M., and Zhengi, J.Q. (2000). Natural Transfer of Viable Microbes in Space: 1. From Mars to Earth and Earth to Mars. *Icarus*, 145, 2, 391-427.

REFERENCES

Callahan, M.P., Smith, K.E., Cleaves II, H.J., Ruzicka, J., Stern, J.C., Glavin, D.P., House, C.H. and Dworkin, J.P. (2011). Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases. *PNAS*, 108, 34 13995-13998.

Clark, B.C., Baker A.L., Cheng, A.F., Clemett, S.J., McKay, D., McSween, H.Y., Pieters, C.M., Thomas, P., and Zolensky, M. (1999). Survival of Life on Asteroids, Comets and Other Small Bodies. *Origins of life and evolution of the biosphere*, 29, 5, 521–545.

Horneck, G., Klaus, D.M., and Mancinelli, R.L. (2010). Space Microbiology. *Microbiology and Molecular Biology Reviews*, 121–156.

Kminek, G., Conley, C., Hipkin, V., Yano, H. (2017) COSPAR's Planetary Protection Policy, *Space Research Today*, #200.

Chapter 3. Lessons learned – How to comply with Planetary Protection Standards

The Lessons learned chapter highlights the most common mistakes and failures occurring during the implementation planetary protection requirements and how to avoid them by understanding the “dos and don’ts” in Planetary Protection. This chapter results from a set of interviews of international planetary protection experts from both the public and private sectors who shared their experience on the most common problems, consequences and possible solutions.

The list of missions/instruments reported on in this chapter includes: Pioneer, Mariner, Viking, Mars Pathfinder (MPF), Mars Exploration Rover, Beagle 2, Europa Orbiter, Mars Reconnaissance Orbiter (MRO), Mars Science Laboratory (MSL), Juno, InSight, ExoMars 2016/2020, MOMA and DREAMS.

COMMON PROBLEMS IN THE CHAIN OF COMMAND (MANAGEMENT/COORDINATION)

PROBLEM 1: AN UNCLEAR CHAIN OF COMMAND WITH PART OF THE CHAIN LEFT WITHOUT A LINK TO THE PLANETARY PROTECTION OFFICER

Consequence: Leads to difficulties in meeting PP requirements.

Solution: Clarifying PP related staffers’ responsibilities throughout the project and organize the team so that one of the PP staffers (either the PPO, or someone from the project or mission or PP team) acts as a link at all stages of PP management, setting in with the PPO, the PP team, the contractors and sub-contractors, helping the easy circulation of information and updates along the project. For Category IV, especially IVb, IVc and V-restricted Earth Return, the PPO should be considered an embedded member of the project PP team and has to be provided with information related to critical spacecraft operations on the planetary surface that may affect PP compliance and/or interests.

PROBLEM 2: PLANETARY PROTECTION RELEVANT PERSONNEL NOT INCLUDED AT KEY ORGANIZATIONAL PROJECT MEETINGS

Consequence: Created conflicts at all levels between scientists, engineers, technicians, and management and difficulties in following compliance with PP requirements.

Solution: Planning an early on meeting with all PP related personnel involvement at all levels of the chain of command ((PPO; Program management; project management; engineers; contamination control personnel and project PP team) to coordinate PP efforts throughout the project and secure strong, clear and positive working relationships.

PROBLEM 3: LACK OF PP SUPERVISION ALONG THE ENTIRE PROJECT

Consequence: Miscommunication and difficulties in handling contingencies.

Solution: Planning regular meetings between the PPO, the Program management, the Project Management and the head of the PP team. Should be reviewed: a) The documentation concerning hardware requiring high levels of bioburden control; b) The schedule for the various PP trainings to put into place for the project staff; c) records for handling hardware.

PROBLEM 4: LACK OF LINKS BETWEEN THE DIFFERENT LEVELS MANAGING PP REQUIREMENTS

Consequence: Miscommunication leading to delays and difficulties in reaching PP requirements.

Solution: Having someone from the PP team as designated link with the PPO, Project team, Program management, engineering team and contamination control team, sitting in all related meetings, to ensure communication throughout the entire mission and avoid surprises, communicate PP concerns, inform of

PP requirements and follow up on the general PP status of the mission. For large projects, this designated link could also be in charge of managing PP training for project personnel as well as review of documentation. For large projects, this is likely to be a full time job.

PROBLEM 5: OUT OF THE LOOP SUB-CONTRACTORS

Consequence: Design flaws as the hardware and other parts couldn't handle DHMR sterilization processing.

Solution: Including sub-contractors in early project planning conversations/ invite them to PP project meetings, overseen by the PPO. Needs to be done in parallel to a Contamination Control approach; sub-contractors should have to implement a CC plan.

PROBLEM 6: LACK OF COORDINATION BETWEEN PROJECT PP TEAM AND QA/QC STAFF

Consequence: Difficulties in matching PP requirements.

Solution: Including QA/QC personnel to early PP Project meetings to facilitate awareness of handling constraints and additional scrutiny of hardware processing activities.

PROBLEMS ON WHEN/HOW TO IMPLEMENT PLANETARY PROTECTION REQUIREMENTS

PROBLEM 7: GREAT VARIABILITY IN WHERE THE PP FUNCTION LAYS /RESIDES WITHIN A PROJECT

Consequence: Difficulty to reach PP requirement compliance.

Solution: Defining titles and PP responsibilities better, at all levels (Space agency; project; contractor).

PROBLEM 8: PLANETARY PROTECTION NOT TAKEN INTO ACCOUNT DURING MISSION DESIGN

Consequence: The selected material may not handle PP sterilization methods forcing to start over, costing money and severe delays to the entire project.

Solution: Considering from the beginning that PP affects mission design. It is consequently important to discuss hardware design and material selection with the PPO at the beginning of the project.

COMMON PROBLEMS DUE TO LACK OF TRAINING

PROBLEM 9: A PROJECT MANAGER, MISSION MANAGER OR EVEN PP TEAM LEAD WITH NO PREVIOUS SOLID PP TRAINING

Consequence: Difficulties in understanding and therefore handling PP requirements at the right stage within the project, ultimately leading to not matching required PP requirements.

Solution: Peer training. A competent PP lead needs to have been the assistant of a competent PP lead in the past (general trainings are not enough in this case). If such competence is lacking, it is important for the PP staffer to follow a more specific training, taught by past PP leads (as is currently the case through NASA and ESA Planetary Protection trainings). Ask your PPO how/when such training could be put into place.

PROBLEM 10: NOT ENOUGH SPECIFIC TRAINING

Consequence: PP is not taught correctly at certain levels of the chain of command, leaving management staffers often with no deep comprehension of PP's utility, while some sub-contractors needing more hands-on trainings would only be provided with theoretical courses.

Solution: Creating a range of trainings adapted to the different ways PP is implemented throughout the project depending on the mission's category ranking (ranging from leading general trainings for management with incentive on the historical role and fundamental purpose of PP to trainings with a hands-on empathy for engineers and designers). These practical trainings should be held where the planetary protection requirements have to be applied to valid the consumables and procedures).

PROBLEM 11: CONTRACTORS NOT AWARE OF WHAT PP IS AND HOW IT MAY INFLUENCE THEIR TASK

Consequence: PP manager will have to spend an important amount of time with contractors to fix problems that could have been avoided. That time spent costs possibilities for the PP manager to link with other parts of project teams that need him. The material used without taking into account PP requirement might also not handle sterilization methods such as DHMR and need to be re-designed.

Solution: Planning that the contractors involved in the spacecraft building phase will get a formal PP training. This way, the design and development of landers/rovers/Hardware will take into account PP requirements from the very start, so the material will be able to resist required sterilization/decontamination temperatures. This can be planned with the help of the (PPO), project manager and PP Lead at the beginning of the project, to help with material selection (that could tolerate DHMR and + other sterilization methods) so, fit PP requirements).

BUDGET OVERRUNS

PROBLEM 12: PROJECT FINANCIERS NOT AWARE OF PP REQUIREMENTS

Consequence: Mistakes when finalizing the cost, resource and AIT/ATLO plans.

Solution: Early on in the project teaching and training PP Financial and schedule aspects to project managers, supervisors and funding agencies.

PROBLEM 13: DIFFICULTY IN ESTIMATING AN EXACT PP BUDGET, WITH CHALLENGES IN ACCOUNTING PERIPHERAL EXPENSE.

Consequence: If a clear PP budget is not set and money is overrun during the course of the project, cuts may occur (lack of training, unsuited sterilization facilities etc.), which will threaten compliance to PP requirements.

Solution: Consensus: plan for ~the price of one instrument of the total project's cost.

PROBLEM 14: COSTS FOR OPERATING THE CLEANROOM AND GARMENTS, ADDITIONAL AND SPECIAL TOOLS AND LAB INSTRUMENTS,

CONSUMABLES, PERSONNEL ETC. WERE MASSIVELY UNDERESTIMATED.

Consequence: Overrun budget due to additional resources and workforce needed.

Solution: Discussing PP cost for cleanrooms and material by establishing a clear PP budget with the PPO at the beginning of the project.

DELAYS

PROBLEM 14: TIME FOR ANY CONTINGENCIES IS NOT TAKEN INTO ACCOUNT WHEN PLANNING THE PROJECT PP CALENDAR.

Consequence: Create over-time translated into over cost and delayed launch.

Solution: Planning extra PP reviews time slots in the calendar for contingencies (and warn the personnel that they happen more often than not and have to be taken into consideration seriously).

PROBLEM 15: WORKING TIME IN CLEANROOMS STRONGLY UNDERESTIMATED (DUE TO THE DIFFERENT PHASES: CLEANING/ASSEMBLY/ TESTING BUT ALSO DUE TO STAFF BEHAVIOR. STAFF NEED TO ADAPT TO THE REGULATORY PROCEDURES WITHIN CLEANROOMS TO MAKE SURE NO RE-CONTAMINATION OCCURS.

Consequence: Delays and possible accidental recontamination.

Solution: Planning working time in cleanrooms with PPO + necessity to dimension the right need for staff and insert in early PP calendar + take into consideration staff rotation. (risk of recontamination by clean room cleaning personnel.

PROBLEM 16: UNDERESTIMATED THE PACKING OF HARDWARE DIFFICULTY FOR TRANSPORT AND TESTS

Consequence: Delays.

Solution: Taking into consideration the packing of hardware preliminary measures during the design phase. The PP lead should advise the sub-contractor how to pack the hardware.

PROBLEM 17: WHEN DESIGNS CHANGE, PP REQUIREMENTS MAY/WILL CHANGE.

Consequence: As PP needs to be set at the beginning of the design chain, a change in the project could mean very costly changes, having to start over or re-design some of the PP procedures.

Solution: Talking from the beginning if some changes may happen or are being considered so alternative PP procedures can be prepared in advance and avoid over costs or delays. + PP lead being part of the change control board/activity.

COMMON ERRORS**PROBLEM 18: OVERLAP/MISUNDERSTANDING/INTERFERENCE BETWEEN PP AND CC REQUIREMENTS.**

Consequence: Leads to confusion between the two.

Solution: Explaining clear difference between PP and CC (possibly through cartoon -like scheme to visualize the differences) or joining them to one approach, such as “cleanliness”. There is a difficulty to mix all types of contaminants even more so as the measurements techniques are really different; Particulate Contamination and Molecular Contamination should be treated together (monitoring often combined).

PROBLEM 19: SHORT-STAFFED PP TEAMS. (RIGHT AMOUNT NOT REQUESTED OR NOT APPROVED BY PROJECT MANAGER)

Consequence: Critical details go unchecked with staff missing critical meetings where they should have been informed of change in hardware for example, bringing later to deficiencies in documentation and difficulties in reaching PP requirements.

Solution: Establishing with Project manager PPO the appropriate number of staff needed for the embedded PP team.

PROBLEM 20: WRONG CHOICE OF TEST FACILITIES.

Consequence: Some of the test facilities (EMC, shock, vibration...) are not in a clean

environment and require additional efforts to keep the hardware clean.

Solution: Reviewing test facilities with PPO at the beginning of the project. The project manager needs to make sure that the needs requested by the PP lead are matched in the contracts about specifications procurements. An acceptance review is needed because sometimes, facilities cannot be cleaned up to the right cleanliness level.

PROBLEM 21: WRONG SELECTION OF MATERIAL.

Consequence: Some materials did not survive the ultra-cleaning process and either the material or process had to be modified.

Solution: Addressing the PP lead for selection of material.

PROBLEM 22: TRAINING BEING PLANNED TOO EARLY OR TOO LATE ALONG THE PROJECT.

Consequence: Trainees training too early have no clear recollection on how to operate well in the cleanroom. Same effect on trainees trained in a rush, too late during the project. No time was planned for adaptation behavior needed for PP staffers to get used to working in cleanrooms.

Solution: Planning trainings early on with PP Lead and Project Manager Officer.

PROBLEM 23: STAND-ALONE PLANETARY PROTECTION DOCUMENTATION.

Consequence: Difficulties in tracking the progress of PP requirement implementation throughout the project.

Solution: PP documentation should be part of the overall project level documentation system, including the formal system to establish and track project level documentation from the program manager to have PP requirements reflected within the level – documentation.

Chapter 4. Planetary Protection Check List

The Check List chapter serves as a general summary of good practices and recommendations.

PLANETARY PROTECTION CHECK LIST

PLAN A FIRST MEETING WITH YOUR PPO DURING WHICH YOU WILL:

1. Establish clear planetary protection roles and responsibilities.
2. Designate dedicated and experienced/trained PP staff at all levels (space agency, project and contractors). Discuss every PP staffer's exact role and responsibilities throughout the project. Review the personnel competence, checking their previous involvements assisting a competent PP lead. Considering their various levels of competence, plan for your staff to attend mandatory PP training adequate to their knowledge and stages in the project in which they will intervene.
3. Discuss the different levels of training with different levels of specialization from manager to on deck staff, taking into account that some staffers will need time to adapt their behavior to strict cleanroom behavioral requirements. Do not forget to consider training your project contractors if they are involved with building elements on which PP requirements may apply. Thus, the material that will be able to resist required sterilization/decontamination temperatures.

Training opportunities may include:

- For managers: Plan mandatory trainings in order to teach PP financial and schedule aspects to project managers, supervisors and funding agencies.
 - For sub-contractors: Plan mandatory hands-on application and implementation-oriented trainings for sub-contractors.
 - Regular short repetition of all trainings in a small group (> 4) (e.g., every 2 years).
- In case of mission delays leading to a substantial amount of hardware engineering personnel turnover, it is important not to forget to train the new recruits on PP protocols to avoid new mistakes resulting in problems meeting the PP requirements for launch approval.

NEXT, PLAN AN EARLY PP MEETING WITH ALL AGENTS INVOLVED WITHIN THE MISSION.

PP meeting participants will include the PPO, program executive, program management, project management, program scientist, engineers, contamination control personnel, project PP team and sub-contractors lead.

DESIGNATE ONE PERSON PER PROJECT TO CARRY THE RESPONSIBILITY OF ACTING AS THE DESIGNATED LINK REGARDING PP ISSUES AT ALL STAGES OF THE PROJECT.

That person should be sitting in all PP related meetings with sub-contractors, program and mission management, the PPO, the Project team, engineering team, QA/QC (Quality Assurance/Quality Control) personnel and contamination control team. That person will ensure communication throughout the entire mission, avoid surprises, communicate PP concerns, inform of PP requirements and follow up on the general PP status of the mission. For large projects, this designated link could also be in charge of managing PP training for project personnel as well as reviewing PP documentation. For large projects, this is likely to be a full time job.

DISCUSS TECHNICAL ASPECTS SUCH AS:

- Material selection and hardware design so it can tolerate PP sampling and DHMR and match PP temperature compliances (the material needs to be able to resist required sterilization/decontamination temperatures).
- Cleanroom environment and protocols (procedure, personnel, monitoring plan)

and flowchart for sampling plan at various locations inside the cleanroom.

DISCUSS THE PP BUDGET

The PP budget shall take into consideration the needs in personnel, various levels of trainings and prices of selected sterilization facilities and material.

PLAN THE PP PROJECT SCHEDULE, FEATURING:

- Training dates
- Testing dates
- Regular meetings between the PPO, the Program management, the Project Management and the head of the PP team.
- Reviews with the PPO to oversee implementation
- Date by which documentation needs to be filled out to match project deadlines
- A timeline taking into account sterilization process times (sterilization facilities need to be booked early)
- An estimate working time in cleanrooms (discussed with locals); Do not forget to plan for the 72-hr bioburden count
- PP reviews/progress meetings with the PPO in-between formal project reviews
- Extra PP reviews for avoid contingencies (PPO, Project manager)

TAKE INTO ACCOUNT THAT THERE WILL BE, MORE OFTEN THAN NOT, CONTINGENCIES

Contingencies can be due to, for example, changes in hardware, or longer than expected bioburden controlled assembly and testing. This should be taken into consideration when preparing the staff's PP calendar. It is therefore necessary to plan extra PP reviews time slots in the calendar and warn the staff, from the very start, that such contingencies are extremely frequent.

DISCUSS POSSIBLE CHANGES, SUCH AS IF HARDWARE REDESIGN IS BEING CONSIDERED. THIS WAY, ALTERNATIVE PP PROCEDURES CAN BE PREPARED IN ADVANCE AND WILL AVOID OVER COSTS OR DELAYS.

- Discuss staffing and workforce
- Accommodate verification essays

FOR CATEGORY IV AND V MISSIONS

- For all Category IV missions: Mandatory PP trainings for ATLO (Assembly, Test and Launches Operations) personnel.
- For Categories IVb and IVc missions, identify a fallback landing site compatible with a PP category IVa implementation (that would satisfy a significant fraction of the science requirements).
- For category IV, especially IV b, IVc and V-restricted Earth Return, the PPO shall be considered an embedded member of the project PP team and has to be provided with information related to critical spacecraft operations on the planetary surface that may affect PP compliance and/or interests.

WORKING WITH YOUR PLANETARY PROTECTION OFFICER

When project includes PP constraints on scientific operations, most managerial difficulties come from the fact that PP relevant personnel is not included at key organizational meetings, which may create conflict at all levels.

Fruitful exchanges with the PPO lead to the better editing of deliverables and a better representation of the areas that were sampled through the use of Computer Aided Design (CAD) technical tools, like on DREAMS.

CONTACT YOUR PPO TO:

- Discuss the budget: taking into consideration needs in personnel (determine the sufficient number of staffers needed for the embedded PP team working throughout the project), various levels of trainings and prices of selected sterilization facilities and material.
- Plan different sorts of trainings (for managers; supervisors; funding agencies; technicians; contractors etc.)
- Select facilities and plan individual tests (taking into consideration time and equipment for re-contamination control during environmental testing)
- Estimate necessary working time in cleanrooms.

- Plan calibration after the Dry Heat sterilization process, as it can impact shipment and delivery sequence.
- Review documentation and records concerning handling hardware that require high levels of bioburden control

CONTACT YOUR PPO FOR:

- Observation of operations affecting PP compliance
- Verification essays
- Project meetings
- All “life detection” experiment meetings with the mission management.
- Documentation reviews (PP documentation is to be considered as part of the overall Project-level documentation system).
- PP reviews & progress meetings in-between formal project reviews
- Launch and landing

GENERAL RECOMMENDATIONS

- Implementation of PP requirements should be accomplished as a system-level process, with PP requirements translated into appropriately flowed-down technical specifications.
- If Planetary Protection affects mission design:
 - Typical compatibility problems with bioburden assay or sterilization process approaches should be addressed as early as the design stage (discuss optical coatings, thermal paints, and general CTE mismatch).
 - DHMR, bio sampling, cleaning compatibility and the packing of hardware need to be design drivers.
- PP requirements impact ATLO (assembly, integration and test) activities and material selection (because of DHMR) (ceramics vs. plastic, lubricants, glues, magnets, laser window...), especially for components with different expansion coefficients.
- In case of secondary payload, a good communication between the secondary payload team and the launch vehicle providers is essential to ensure that planetary protection

requirements on the primary payload are not violated.

- Put into place frequent and even informal communication to easily maintain PP efforts along the project chain of management.

TO AVOID DELAYS:

- Plan time for hot and dry sterilization processing as well as the waiting time for the results on the cultures (24-72hrs).
- Take into consideration that Bioburden controlled assembly and testing takes substantially longer than originally planned.
- Working time in cleanrooms is often underestimated. Staffers will need time to get used to strict specific cleanroom behavioral requirements. Make sure to discuss it with your PPO early on, to be taken into consideration when building the PP calendar.

Annexes

This section provides some insight on how major space agencies including ESA, NASA and JAXA manage planetary protection for their planetary exploration missions.

Planetary Protection at ESA³⁰

ESA PLANETARY PROTECTION POLICY

RATIONALE FOR A POLICY

- Based on Article II of the ESA Convention, the Agency is acting on behalf of its Member States
- Execution of activities and programs by ESA shall therefore be consistent with the Member States rights and obligations pursuant to international agreements, including the UN Outer Space Treaty
- The ESA Planetary Protection Policy is intended as an essential tool for ensuring that necessary means are made available to avoid interplanetary contamination when the Agency is carrying out activities in outer space, mindful of Member States' corresponding obligations

MAIN POLICY STATEMENT

- This “ESA Planetary Protection Policy”, complies with the COSPAR planetary protection policy and the corresponding implementation guidelines
- Spaceflight missions carried out with any degree of ESA involvement shall comply with this policy and its associated requirements

AUTHORITY LEVEL

- ESA Council, document reference ESA/C(2007)112
- Any revision is subject to approval by the ESA Council

ESA PLANETARY PROTECTION REQUIREMENTS

ESA PLANETARY PROTECTION REQUIREMENTS, ESSB-ST-U-001

- In line with the COSPAR Planetary Protection Requirements

Approved by the ESA Standardization Steering Board

SCOPE OF THE DOCUMENT

- The overall planetary protection management requirements
- The technical planetary protection requirements for robotic and human missions (forward and backward contamination)
- The planetary protection requirements related to procedures
- The Document Requirement Descriptions (DRD) and their relation to the respective reviews

APPLICABILITY OF THE DOCUMENT

- ESA spaceflight missions
- Contributions to ESA spaceflight missions
- ESA contributions to non-ESA spaceflight missions

ESA PLANETARY PROTECTION ORGANIZATION

The ESA Planetary Protection Organization is described in Figure 52 below.

³⁰ This section was provided by Dr Gerhard Kmínek.

ESA PLANETARY PROTECTION RESPONSIBILITY

CORPORATE RESPONSIBILITY

- Establish, maintain and act as custodian of the ESA Planetary Protection Requirements;
- Advise and support relevant ESA programmes and projects on matters of planetary protection;

- Approve planetary protection categorization and requirements for flight projects;
- Perform assessments including inspections and reviews of facilities, equipment, procedures and practices as appropriate to ensure compliance with the planetary protection requirements;
- Certify the planetary protection compliance in the course of flight projects (part of launch certification).

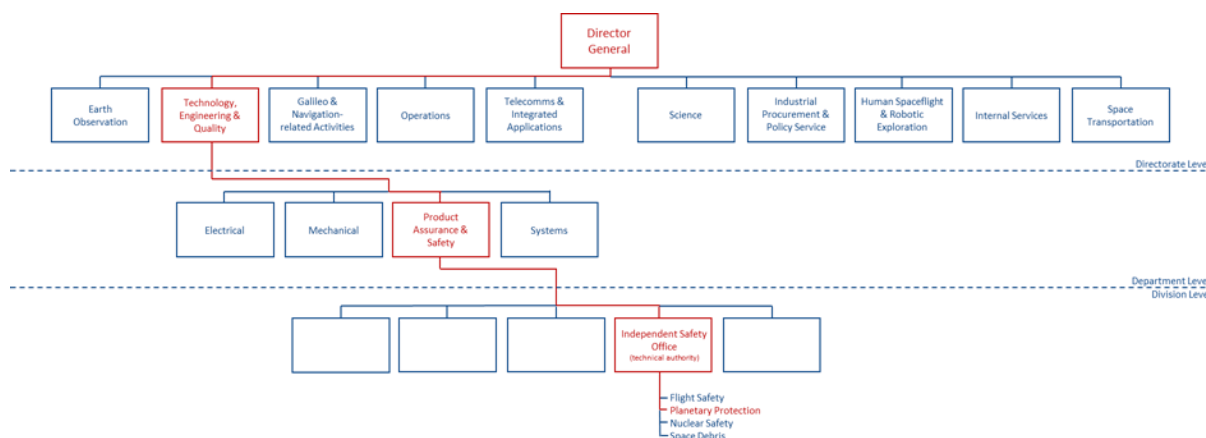


Figure 52 – Organization of Planetary Protection at ESA.

PROJECT RESPONSIBILITY

The Project Manager is responsible for the correct identification and implementation of the planetary protection requirements at project level.

In particular, the Project Manager is responsible to:

- Identify the planetary protection requirements specific to the project by tailoring this standard and relevant planetary protection standards in the list of ESA approved standards;
- Define the planetary protection implementation and management approach;
- Define the planetary protection responsibilities within the project;
- Prepare project-level planetary protection documentation;

- Consider the implementation of the recommendations of reviews with respect to planetary protection aspects.

THINGS TO REMEMBER

- ESA is compliant with the COSPAR Planetary Protection Policy;
- The corporate planetary Protection responsibility is with the Product Assurance & Safety Department;
- The responsibility to correctly implement the planetary protection requirements is with the Project Manager;
- A range of standards are available to support the implementation of the planetary protection requirements.

Planetary Protection at NASA³¹

HISTORICAL DEVELOPMENT

In the early days of the space age, even before the launch of Sputnik, scientists had expressed concern on how planetary exploration should be conducted, with respect to guarding against the threat of contamination. The timeline below reflects the development of the issue, leading up to the appointment of the first planetary protection officer at NASA, Dr. L. Hall:

- September 1956: International Astronautical Federation meets in Rome, discussing lunar and planetary contamination;
- February 1958: International Council for Science (ICSU) forms committee on Contamination by Extra-Terrestrial Exploration (CETEX);
- June 1958: National Academies of Science establishes the Space Studies Board;
- July 1958: Formation of the US National Aeronautics and Space Administration (NASA);
- October 1958: Formation of COSPAR by ICSU;
- July 1958: Formation of UN-COPUOS (Committee on the Peaceful Use of Outer Space);

- 1959-1962: Publication of guidelines by the US, USSR, COSPAR;
- 1963: NASA acquires the first ‘Planetary Quarantine Officer’ – on loan from the Public Health Service.

ORGANIZATION OF PLANETARY PROTECTION WITHIN NASA

Even from this early time, before the first Apollo launches had begun, the Space Science Board (predecessor to the Space Studies Board of the National Academies of Sciences) was providing advice to NASA on the conduct of the exploration of Mars. For example, that NASA should “*Accord the highest priority to the prevention of the biological contamination of Mars until sufficient information has been obtained about possible life forms there so that further scientific studies will not be jeopardized*” was early advice to the new PPO from the Space Science Board in August 1963.

It should be noted that this exhortation pre-dates knowledge of Europa and Enceladus as bodies of significant interest with respect to chemical evolution/origin of life in the solar system, and in the context of this volume, they are now similarly regarded in how NASA planetary protection policy treats different solar system targets.

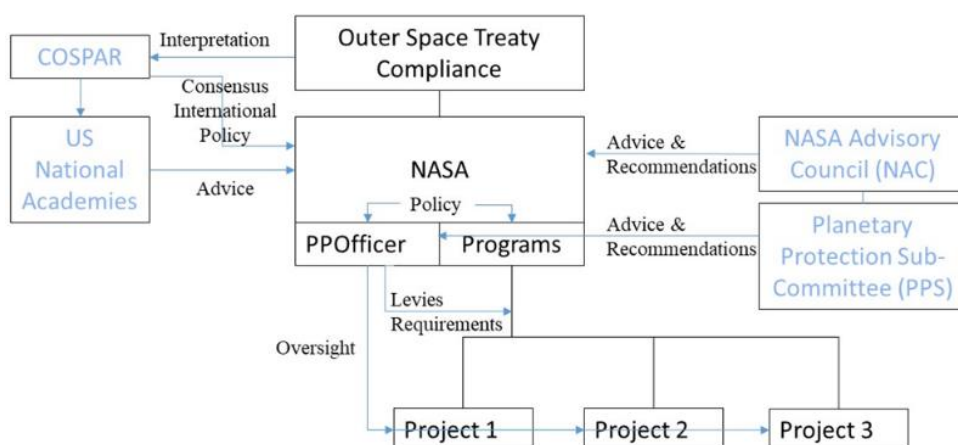


Figure 53 – Notional paths for information flow on planetary protection issues for NASA missions.

It also pre-dates the 1967 Outer Space Treaty which, in the current timeframe, is the law of

the land in the USA with which NASA has to demonstrate compliance (Figure 53). NASA

³¹ Section based on a presentation by Andy Spry (SETI).

also receives advice and recommendations from its own advisory council (NAC) of which the Planetary Protection Sub-committee has been the primary non-NASA reviewer of planetary protection implementation practices for NASA missions.

DOCUMENT HIERARCHY

Within NASA is a document hierarchy for planetary protection, to ensure planetary protection compliance for NASA missions:

1. NASA Planetary Protection Policy NPD8020.7: The highest level planetary protection document describing policy goals and authority (the “why” for planetary protection implementation).
2. NASA Planetary Protection Procedural Requirements for Robotic Missions NPR8020.12: Is the requirements document, describing what is needed to demonstrate compliance with Policy goals (the “what” for planetary protection implementation).
3. NASA Technical Standard for Planetary Protection NASA-STD-6022 Protocols and specifications achieve compliance with Policy goals (the “how” for planetary protection implementation).

IMPLEMENTATION HISTORY

NASA’s previous missions show a history of implementation practice and development to achieve compliance with planetary protection policy. The Apollo missions developed a monitoring and quarantine approach for crewed missions. The Vikings developed a terminal system-level sterilization approach that allowed landing a spacecraft on Mars with acceptably low risk of forward contamination.

In contrast, the Galileo and Cassini missions to the Jovian and Saturnian systems, respectively, maintained their risk of contamination by impact avoidance.

MRO, as a result of uncertainties in the execution of its aerocapture maneuver, could not meet the required risk of contamination by impact avoidance alone, and hence utilized bio-burden control and post-launch breakup and

burn-up analyses to demonstrate that it would not cause harmful contamination at Mars.

Similarly, the Juno mission could not maintain a 1×10^{-4} probability of avoidance of impact at Europa, hence the bio-burden of that mission had to be managed, but addressing the bio-burden reduction effects of the jovian radiation environment and the heating effects of a potential impact at Europa to demonstrate the avoidance of contamination at the required level.

For future missions, NASA policy describes requirements for Icy Moons (From NPR8020.12D) as follows: “*Requirements for flybys, orbiters, and landers to Icy Satellites, including microbial reduction, shall be applied in order to reduce the probability of inadvertent contamination of an ocean or other liquid water body to less than 1×10^{-4} per mission. These requirements will be refined in future years, but the calculation of this probability should include a conservative estimate of poorly known parameters and address the following factors, at a minimum:*

- *Bioburden at launch.*
- *Cruise survival for contaminating organisms.*
- *Organism survival in the radiation environment adjacent to the target.*
- *Probability of encountering/landing on the target, including spacecraft reliability.*
- *Probability of surviving landing/impact on the target.*
- *Mechanisms and timescales of transport to the subsurface.*
- *Organism survival and proliferation before, during, and after subsurface transfer.*

Preliminary calculations of the probability of contamination suggest that microbial reduction will likely be necessary for PP Category III orbiters as well as for PP Category IV landers. This will require the use of cleanroom technology, the cleanliness of all parts before assembly, and the monitoring of spacecraft assembly facilities to understand the bioload and its microbial diversity, including specific problematic species. Specific methods should be developed to eradicate problematic species.”

In developing a general approach for missions to the Jovian system where impact avoidance alone is insufficient to achieve compliance, it is necessary to take into account the differential survival of different sub-populations of the bioburden. Following on from the recommendations of the SSB report “Preventing the Forward Contamination of Europa”, the JIMO (Jupiter Icy Moons Orbiter) project and successor studies partitioned the spacecraft bioburden into four sub-population types, categorized as in Figure 54:

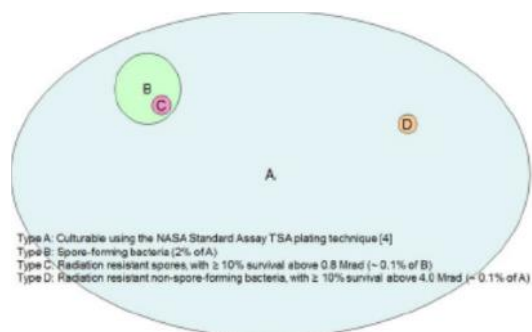


Figure 54 – Spacecraft bioburden categories in the JIMO project and successor studies.

These categories could then be assessed as four different disjoint groups, for accounting purposes (Table 17).

Finally, in the analysis, these groups could then be enumerated and recombined to give surviving bioburden as an input to the overall probability of contamination calculation (Figure 55).

Group	Type	Definition
1	A–B–C	Radiation sensitive non-spore-forming bacteria*
2	B–C	Radiation sensitive spore-forming bacteria*
3	C	Radiation resistant spore-forming bacteria*
4	D	Radiation resistant non-spore-forming bacteria*

* culturable using the NASA Standard Assay TSA plating technique

Table 17 – Groupings of bioburden types for bioburden accounting purposes in JIMO and successor studies.



Figure 55 – Bioburden accounting by bioburden type through a mission study timeline.

In summary, NASA has maintained compliance with the Outer Space Treaty by following COSPAR Policy. The NASA PPO levies requirements on projects via usual project requirements flow-down, then monitors compliance. NASA receives independent advice and guidance to maintain up to date policies and practice. Projects interpret and implement requirements, developing mission-specific (and sometimes novel) strategies to achieve compliance at time of launch.

Planetary protection management in JAXA³²

Until ~2003 in Japan there were two space agencies which could launch rockets:

- The Institute of Space and Astronautical Science (ISAS) which was responsible for spacecraft for science.
- The National Space Agency of Japan (NASDA) which was responsible for spacecraft utilization (broadcast, earth observation, engineering tests).

ISAS was established as an institute of Tokyo University. It was directly controlled by the Ministry of Education. It participated in missions to Comet Halley since 1981. The Sagami-hara campus was established in 1989. ISAS researchers are involved in education as professors. Graduate students and postdocs from universities are involved in ISAS programs. ISAS research departments encompassed both science and engineering. It operated a launch field at Uchino-ura from which solid fuel rockets were launched (e.g., M-V in the 1990s).



Figure 56 – Launch of an H-II rocket (2 tons, 7-8 tons max.) to the Moon on 4 February 1994 (Credit: JAXA)

NASDA was established in the 1960s, for space utilization and development under Japan's national policy. NASDA had more than 1,000 employees, most of which were engineers. It operated a launch field on Tanegashima Island, from which were launched rockets using liquid H and O (e.g., the H-II rocket, Figure 56). NASDA launched

Earth observation satellites and modules for the Space Station (astronauts). NASDA also owned the Tsukuba center (test facilities, research center), and other facilities.

In 1996 a Working Group started design studies of the SELENE project, which was officially approved in 1998 as a joint program of NASDA and ISAS.



Figure 57 – SELENE (Kaguya) (Credit: JAXA)

The Japan Aerospace Exploration Agency JAXA was founded in 2003 through the unification of ISAS, NASDA and NAL, the National Aerospace Laboratory. The SELENE (Kaguya) mission was “a symbol of the unification” (Figure 57).

There is a sharing of responsibilities between JAXA and ISAS. ISAS proposes, develops and operates missions possibly relating to planetary protection. JAXA is responsible for planetary protection. The overall Planetary Protection organization of JAXA is represented in Table 18.

The Department of Safety and Mission Assurance (S&MA) establishes and maintains ‘standards’ for planetary protection. It organizes a planetary protection standardizing Working Group and supports its work. The Chair of the WG is K. Fujita (JAXA).

The International Space Exploration Team has responsibility for establishment of PP strategy and storage of information and technologies along it, with organizing “PP research group”. Chief of the group is J. Haruyama (tentative).

³² This section is based on a presentation by Junichi Haruyama from ISAS/JAXA.

The Planetary Protection Review Board reviews PP of projects and/or relevant matters.

The Chair of the board is A. Yamagishi (Tokyo University of Pharmacy and Life Sciences).

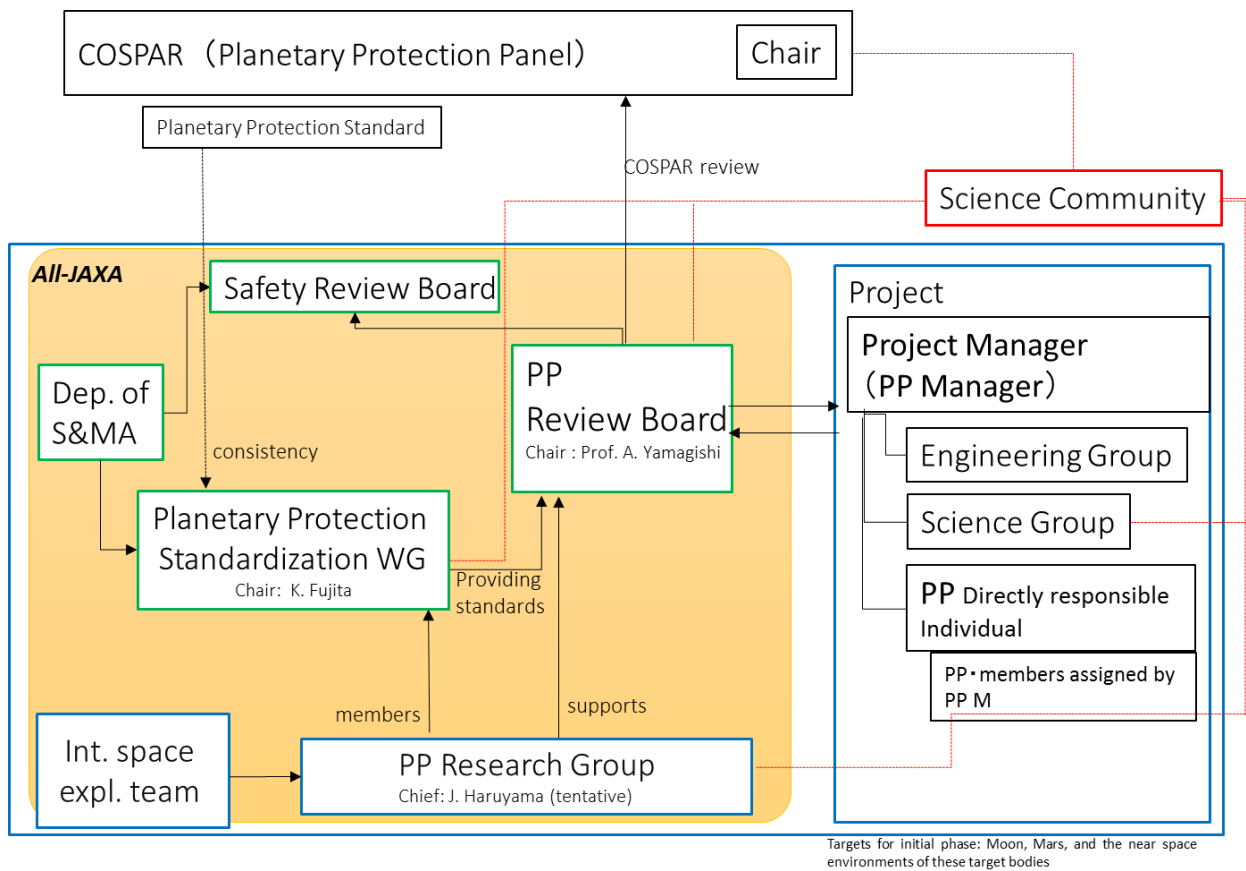


Table 18 – “All-JAXA” planetary protection organization diagram (in the beginning)

NOTES

NOTES



Committee on Space Research (COSPAR)

2 Place Maurice-Quentin, 75001 Paris, France

500 rue Jean-François Breton, 34090 Montpellier, France

Phone: +33(0)1 4476 7441 / +33(0)4 6754 8727

Email: cospar@cosparhq.cnes.fr

COSPAR Web site: www.cosparhq.cnes.fr

PPOSS Web site: www.pposs.org