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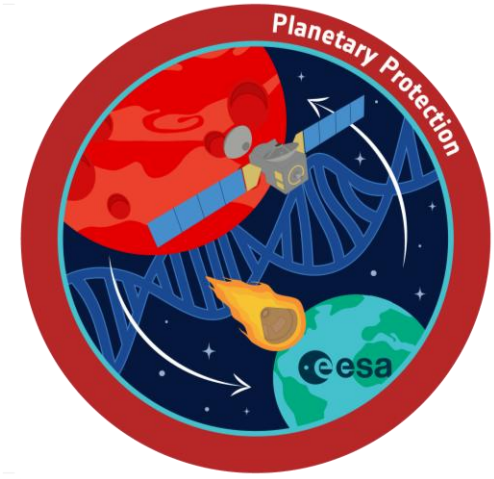
ESA PLANETARY PROTECTION REQUIREMENTS COMPLIANCE VERIFICATION HANDBOOK



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Introduction

Planetary Protection is the discipline of promoting sustainable and responsible space exploration by tackling the potential transfer of biological matter to and from Earth and other objects in the Solar System. Although conditions on other planets and bodies in space look inhospitable, ingredients that can host life can be present. If life exists in any of the worlds we hope to explore, mission teams cannot leave its protection to chance.

The legal international basis for Planetary Protection was established in Articles VI and 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 (Outer Space Treaty) [RD17].

The ESA regulatory framework for Planetary Protection was formally created in 2007, with the release of the "ESA Planetary Protection Policy", ESA/C (2007)112, and its approval by ESA Council. Since then, spaceflight missions carried out with any degree of ESA involvement comply with this Policy.

The ESA Planetary Protection Policy is fully guided by the COSPAR Planetary Protection Policy [RD02] and the corresponding implementation guidelines. It is based on 2 rationales:

a. Ensure that scientific investigations related to the chemical evolution and origin of life in the Solar system are not compromised.

b. Protect the Earth from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from an interplanetary mission.

A set of Planetary Protection requirements has been created and made applicable to ESA space missions to ensure compliance with ESA/C (2007)112. These requirements are contained in ECSS-U-ST-20 (2019) and include 80 individual requirements and 8 DRDs (Document Requirements Definitions). However, there is no information available to the projects on the rationale for the adopted ECSS Planetary Protection requirements and the approach to their verification, hence there is the need for a Planetary Protection handbook.

1 Scope

This handbook provides guidelines on verification methods and possible implementation of measures in support of ESA Projects to facilitate the compliance with the ESA Planetary Protection (PP) requirements defined by the ESA PP policy ESA/C/(2007)112 [RD1], COSPAR PP Policy 2021 [RD2] and the standard ECSS-U-ST-20.

This document has been prepared by the ESA Planetary Protection Working Group, convened and coordinated by ESA Planetary Protection Officer (PPO), involving experts from the relevant disciplines in the ESA Technical, Engineering and Quality (TEC) Directorate, ESA Operations (OPS), Human and Robotic Explorations (HRE) and Science (SCI) Directorates.

This handbook also provides a description of the analysis, approaches and documentation, which are prepared to demonstrate compliance with ESA Planetary Protection requirements.

The intended users of this handbook are all ESA projects and their partners, including commercial and private entities, ESA Directors, ESA Project Managers, Study Managers, Mission Managers, Product Assurance and Safety Managers, System Engineers, experts and all technical personnel, involved in the design or operation control of space systems with ESA Planetary Protection requirements.

Compliance with the guidelines contained in this handbook does not automatically give approval of compliance with specific project Planetary Protection requirements, which is instead given by the ESA Planetary Protection Approval Authority (PPAA), as defined in [RD4].

This handbook is a live document and a tutorial. The periodical update is foreseen as scientific knowledge and methodologies evolve.

2 References

[RD1]	ESA Planetary Protection Policy [ESA/C/(2007)112]
[RD2]	COSPAR Planetary Protection Policy June 2021
[RD3]	ECSS-U-ST-20 Planetary Protection Requirements – latest revision applies
[RD4]	ESA-TECQI-PR-2023-001247 The ESA Planetary Protection Review and approval process – latest revision applies
[RD5]	ECSS-Q-ST-70-01 Cleanliness and contamination control latest revision applies
[RD6]	ECSS-Q-ST-70-53 Materials and hardware compatibility tests for sterilization processes - latest revision applies
[RD7]	ECSS-Q-ST-70-55 Microbial examination of flight hardware and cleanrooms - latest revision applies
[RD8]	ECSS-Q-ST-70-56 Vapour phase bioburden reduction of flight hardware - latest revision applies
[RD9]	ECSS-Q-ST-70-57 Dry heat bioburden reduction of flight hardware - latest revision applies
[RD10]	ECSS-Q-ST-70-58 Bioburden control of cleanrooms - latest revision applies
[RD11]	ESSB LEAS ESA List of Applicable Standards - latest revision applies
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3 Terms, definitions and abbreviated terms

3.1 Terms from other documents

a. For the purpose of this handbook, the terms and definitions from ECSS-U-ST-20 apply, in particular for the following terms:

1. **assay**
2. **controlled conditions**
3. **encapsulated bioburden**
4. **exposed surfaces**
5. **highly controlled**
6. **Mars special region**
7. **organic material**
8. **planetary protection approval authority (PPAA)**
9. **planetart protection category**
10. **safety critical function**
11. **water activity**

b. For the purpose of this handbook, the terms and definitions from ECSS-Q-ST-70-53 apply, in particular for the following terms:

1. **micro-organism**

c. For the purpose of this handbook, the terms and definitions from ECSS-Q-ST-70-55 apply, in particular for the following terms:

1. **bioburden**
2. **biodiversity**

d. For the purpose of this handbook, the terms and definitions from ECSS-Q-ST-70-56 apply, in particular for the following terms:

e. For the purpose of this handbook, the terms and definitions from ECSS-Q-ST-70-57 apply, in particular for the following terms:

f. For the purpose of this handbook, the terms and definitions from ESA-TECQI-PR-2023-001247 apply, in particular for the following terms:

1. **Planetary Protection Officer (PPO)**
2. **Planetary Protection Lead (PPL)**

3.2 Terms specific to this document

3.1.1 ambient humidity

absolute humidity of ≤ 12 g/m^3 , which is equivalent to 70 % relative humidity at 0 °C and 1000 hPa pressure.

3.1.2 dry humidity

absolute humidity of ≤ 1,2 g/m^3, which is equivalent to 25 % relative humidity at 0 °C and 1000 hPa pressure, or to 7 % relative humidity at 20 °C and 1000 hPa pressure.

3.1.3 Earth-Moon System

single environment of the Earth and the Moon, including artificial objects in orbit around either body.

NOTE For sample return from restricted sample return bodies (Mars, Europa, Enceladus, others) Earth-Moon system is considered as single environment.

3.1.4 encapsulated bioburden

bioburden inside the bulk non-metallic materials not manufactured with additive layer manufacturing.

NOTE Examples are bioburden inside paints, conformal coatings, thermal coatings, adhesives, composite materials, closed-cell foam, bulk liquids and bulk gasses.

3.1.5 ESA Planetary Protection Approval Authority (PPAA)

ESA authority to approve the Planetary Protection compliance of spaceflight missions with ESA involvement with the ESA Planetary Protection Policy.

3.1.6 exposed surfaces

internal and external surfaces free for gas exchange.

NOTE False positive. In a life-detection and/or hazard-determination protocol, it is any unwanted organic contamination introduced to the sampling process. As a consequence of cross contamination in the sample under analysis, validity of the sample and associated scientific results can be affected.

3.1.7 extant life

form of life, or signatures thereof, whether metabolically active or dormant.

3.1.8 extinct life

form of life, or signatures thereof, that is unambiguously no longer metabolically active or dormant.

3.1.9 international scientific consensus

scientific opinion of the international space science community that can have impacts on mission design, categorisation and operations through end of life and disposal.

NOTE Sources of information on current scientific consensus include COSPAR PP panel, ESF – European Science Foundation and peer-reviewed scientific journals.

3.1.10 mated surfaces.

surfaces joined by fasteners rather than by adhesives.

3.1.11 non-nominal

scenarios that cover cases where some condition can occur that results in the system performing in a way that it is different from normal.

NOTE This includes failures, low performance, unexpected environmental conditions, or operator errors that can affect compliance with the probabilistic guidelines.

3.1.12 organic archive

complete inventory with included organic products that can be released into the environment of the protected Solar system body by propulsion and life support systems.

NOTE Inventory include a quantitative and qualitative description of major chemical constituents and the integrated quantity of minor chemical constituents present.

3.1.13 period of biological exploration

time, decades to centuries, during which a Solar system body is explored for signs of the origin of life and the history of prebiotic chemistry based on current scientific understanding.

3.1.14 planetary protection category

classification used to determine how strictly planetary protection measueres can be to prevent the transfer to the Earth-Moon System of all materials from another habitable world that are not sterilized or contained to Earth's biosphere.

3.1.15 probability of contamination (Pc)

probability of introducing into the environment of a Solar system body unwanted material present on or in the spacecraft.

3.1.16 sample

intentionally collected or unintentionally adhering physical material, including solids, liquids, and gasses, that reach the spacecraft returning to the Earth-Moon system from other Solar system bodies.

3.1.17 special region

region within which terrestrial organisms are likely to replicate.

NOTE 1 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. Spacecraft-induced Special Regions are to be evaluated, consistent with these limits and features, on a case-by-case basis.

NOTE 2 In the absence of specific information, no Special Regions are currently identified on the basis of possible Martian life forms. If and when information becomes available on this subject, Special Regions can be further defined on that basis.

3.1.18 uncontrolled humidity

humidity level that is either not measured or does not conform to ambient or dry humidity.

NOTE It is important to note that: Ambient ≠ uncontrolled.

3.1.19 water activity

ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature.

3.3 Abbreviated terms

Abbreviation	Meaning
AED	approach entry descent
AIT	assembly, integration and testing
ALM	additive layer manufacturing
BAT	best available techniques
BI	biological indicator
BuBu analysis	break up and burn analysis
CEA	chemical equilibrium with applications
CC	cleanliness and contamination
CC&C	cleanliness and contamination control
CCRS	capture, containment and return system
CDR	critical design review
CFRP	carbon fibres reinforced materials
CFU	colonies forming units
COSPAR PP	Committee on space research planetary protection
COSPAR PPP	Committee on space research planetary protection panel
CTE	coefficient of thermal expansion
CVCM	collected volatile condensable material
DALY	disability-adjusted life year
ddPCR	digital droplet polymerase chain reaction
DNA	deoxyribonucleic acid
DHMR	dry heat microbial reduction
DML	declared materials list
DMPL	declared mechanical parts list
DPL	declared processes list
DRD	document requirements definition
D-value	decimal reduction - value
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EES	earth entry system
ECSS	European Cooperation for Space Standardization.
EDLM	entry and descend landing module

Abbreviation	Meaning
EMC	electromagnetic compatibility test
ERO	earth Return orbiter
ESCC	European Space Components Coordination
ESSC	European Space Sciences Committee
ESF	European Science Foundation
EU GMP	European Union general manufacturing practices
EQM	engineering qualification model
FMEA	failure mode and effects analysis
FMECA	failure modes, effects, and criticality analysis.
FTA	fault tree analysis
FTIR	fourier transform infrared analysis
GBT	glove box trains
GC-MS	gas chromatography mass spectroscopy
GSE	ground support equipment
Gy	gray
HC	highly controlled
HDBK	handbook
HEPA	high efficiency particulate air
HiRISE	high resolution imaging science experiment.
HVAC	heating ventilation and air conditioning
ICRF	internation celestial reference frame
IPA	iso propyl alcohol
ISO	international organization for standardization
JPL SPICE	jet propulsion laboratory spacecraft planet instrument C-matrix Events
LSSWG	landings site selection working group
MEPAG	Mars exploration program analysis group
MIRT	Mars sample return independent review board response team
MLI	multi-layer insulation
MLS	Mars laboratory science
MOC	Mars launch system
MOLA	Mars orbiter laser altimeter
MSR	Mars sample return
MSR IRB	Mars sample return independent review board
NGS	next generation sequencing
NRC-SSB	national research council space studies board
OD	orbit determination
OIM	orbit insertion module
OR	orbiting sample
PBE	period of biological exploration
PBST	phosphate-buffered saline with tween-20

Abbreviation	Meaning
PFO	particle fall out
PDR	preliminary design review
PPAA	planetary protection approval authority
PPAP	planetary protection advisory panel
PPL	planetary protection lead
PPO	planetary protection officer
PP	planetary protection
PPRSRP	planetary protection re-entry safety review panel
PRR	preliminary readiness review
PSR	permanent shadowed region
PRA	probabilistic risk assessment
PVC	poly vinyl chloride
QM	qualification model
QR	qualification review
RFD	request for deviation
RFW	request for waiver
R2A	reasoner's 2A agar
RML	recovery mass loss
rRNA	ribosomal RNA
SEM	scanning electronic microscope
SRR	shipment readiness review
STPA	systems theoretic process analysis
TML	total mass loss
TOC	total organic carbon
TQCM	thermal quartz crystal microbalance
TRR	test readiness review
TSA	tryptic soy-agar
TVAC	thermal vacuum test
TQCM	thermos quartz crystal microbalance
UCZ	ultra clean zones
UTTR	Utah test and training range
UV	ultraviolet
UV-C	ultraviolet-C
VBNC	viable but not cultivable
VCB	verification control board
UHF	ultra-high vacuum
VHP	vapor hydrogen peroxide

4
Planetary Protection in ESA

4.1 Planetary Protection overview

Planetary protection overarching goal is to ensure that the scientific research related to the chemical evolution and origin of life in our Solar System is not compromised (forward contamination) and to preserve Earth's biosphere from the return of potential detrimental material from outer space (backward contamination)

As space becomes more accessible, new actors are investing in space and planning to launch complex and innovative missions ESA is shaping its role accordingly, with the responsibility to protect the pristine environments of the bodies that are going to be explored; and protect Earth biosphere and public health from potential hazardous extraterrestrial materials.

To meet its objectives, Planetary Protection aims to limit the probability of harmful contamination, when investigating the process of chemical evolution and origin of the Solar system. Although environments of celestial bodies appear hostile, and the probability of Earth microorganims being "harmful" for search for life is extremely low, those conditions do not preclude the possibility of indigenous life forms.

Given current scientific understanding [RD2], [RD3], the limiting physical environmental parameters in terms of water activity and temperature thresholds satisfied at the same time to allow the replication of terrestrial microorganisms are:

- Lower limit for water activity - 0,5.
- Lower limit for temperature - 28° C.

Planetary Protection is about protecting science (hence investements in space), public health and promoting a sustainable and responsible space exploration.

4.2 Governance of Planetary Protection

The mandate for Planetary Protection has been given by the Outer Space Treaty (OST), Articles VI and IX. The legal basis and the goal for Planetary Protection is established in Article IX of OST, which is a complex provision addressing three separates, but interconnected, key principles, namely "due regard", "harmful contamination", and "harmful interference" [RD19].

The "harmful contamination" element connects with the general duty in Article IX for States Parties to conduct space activities "with due regard to the corresponding interests of all other States Parties to the Treaty". In broad terms, States Parties are to ensure that the exercise of their rights and freedoms in outer space does not interfere with or compromise the safety of space activities and operations of other States Parties.

With the consultation requirement on potentially "harmful interference", by Article IX as a whole, States Parties when implementing their treaty obligations on Planetary Protection, are needed to consider the interests of other States Parties in the exploration and use of outer space and consult with other States Parties where these interests can be seriously affected.

Article VI is related to international responsibility for national activities in outer space. It is the prerogative of States to regulate their national space activities.

For Planetary Protection purposes, the combination of Article IX and Article VI of the Outer Space Treaty coupled with the cooperation and coordination mechanism provided by the COSPAR Panel on Planetary Protection, has contributed significantly to the protection against biological contamination and has evolved over the past 60 years into a well-established international framework (as shown in Figure 4-1).

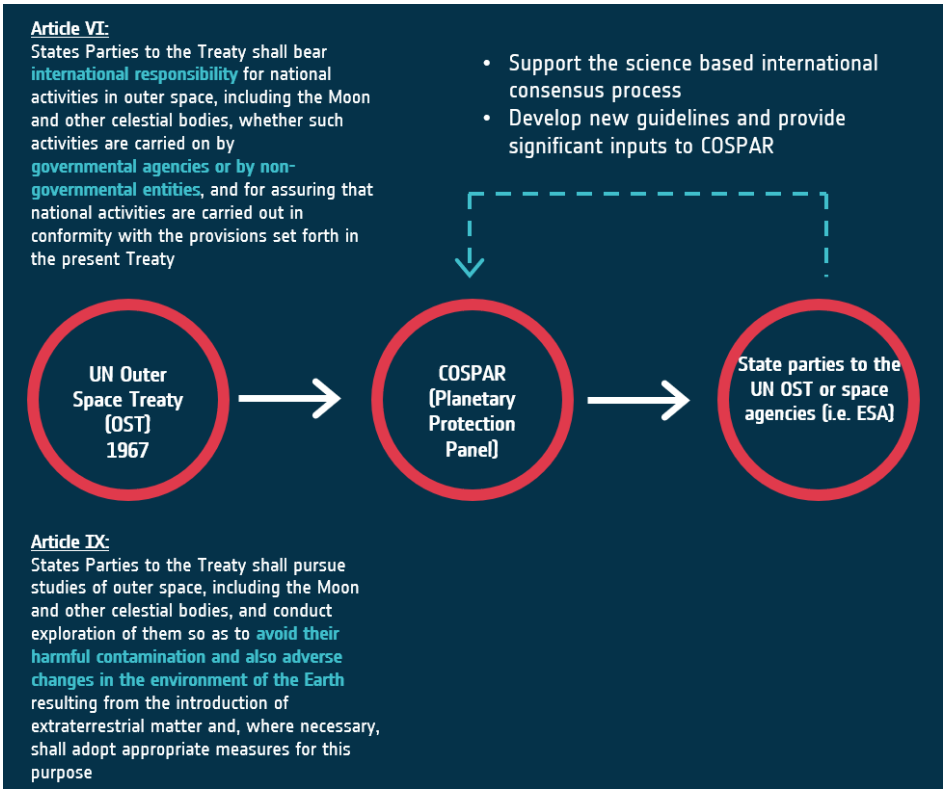


Figure 4-1: Internation framework for Planetary Protection

ESA's Planetary Protection function supports the science based international consensus, by providing significant inputs to the international community at large, including the COSPAR Panel on Planetary Protection as a contribution to develop internationally respected guidelines.

The international framework for Planetary Protection, including the ESA role inside COSPAR PP panel and in supporting international consensus on developing new guidelines, is summarised in Figure 4-1.

4.3 ESA Planetary Protection Policy

ESA have stipulated a Planetary Protection Policy [RD1], which is approved at ESA Council level and it is fully compliant with COSPAR PP Policy [RD2]. The latter contains a set of non-legally binding guidelines to guide compliance with the Outer Space Treaty (OST).

ESA acts on behalf of its Member States, all of which are State parties to the Outer Space Treaty. As such, the execution of ESA's activities comply with the Member States' obligations pursuant to Article IX of the Outer Space Treaty [RD17].

The main objectives of the ESA Planetary Protection Policy (in line with Outer Space Treaty) are:

a. To prevent harmful contamination (biological and organic) in outer space.

b. To protect Earth from inadvertent changes in the environment as a result of sample return missions.

The ESA Planetary Protection Policy is applicable to all spaceflight missions beyond Earth orbit with ESA involvement, including third party contributions to ESA missions.

4.4 Planetary Protection process within ESA

4.4.1 Overview

The roles and responsibilities for the implementation of the PP policy; as well as the process to obtain Planetary Protection approval and certification, are specified in the ESA Planetary Protection review and approval process [RD4].

4.4.2 Roles and responsibilities at ESA

4.4.2.1 Project representatives

Project representatives are the Project Managers, Mission Designers, System Engineers etc. Their responsibilities are:

- Generation of the project PP category and requirements document.
- Definition of the project Planetary Protection implementation and management approach.
- Definition of the Planetary Protection responsibilities within the project and nomination of a Planetary Protection Lead (PPL) within the project.
- Regular exchange with the PPL about the PP status of the project.
- Ensuring the PPL has the resources to fulfil his/her responsibilities.
- Supporting Planetary Protection reviews as necessary.
- For restricted Earth return missions: support of the return segment authorization reviews.
- Reporting of anomalies (pre- and post-launch) to the Planetary Protection Officer (PPO) for consultation regarding the PP compliance.

4.4.2.2 Planetary Protection Lead

The **Planetary Protection Lead** is appointed by project with the following responsibilities:

- Definition of derived Planetary Protection requirements at system and sub-system level, ensuring the flow down of Planetary Protection requirements to relevant sub-contractors including payload providers.
- Ensuring the project implements all PP relevant requirements.
- Ensuring the project generates the PP documentation per the PP requirements document.
- Definition of necessary Planetary Protection operational procedures prior to launch.
- Supporting Planetary Protection reviews as necessary.
- For restricted Earth return missions: documentation generation and support of the return segment authorization reviews.
- Generation and submittal of RFD/RFW for any type of nonconformance with the PP requirements.

- Regular progress exchange with the PPO to ensure the requirements implementation is in line with the intent of the PP requirements.
- Facilitate and coordinate independent PP requirement compliance verifications performed by the PPO.

4.4.2.3 ESA Planetary Protection Approval Authority (PPAA)

The ESA **Planetary Protection Approval Authority** (PPAA) is the ESA authority to approve the Planetary Protection compliance of spaceflight missions with ESA involvement with the ESA Planetary Protection Policy, as per Table 4-1.

Table 4-1: ESA authority for the approval of PP deliverables

<table>
<tr><th rowspan="2">Deliverables</th><th></th><th colspan="5">Category</th></tr>
<tr><th>I</th><th>II</th><th>III</th><th>IV</th><th>V restricted</th><th>V unrestricted</th></tr>
<tr><td>Planetary Protection Category and Requirements</td><td colspan="2">The PPO</td><td colspan="3">The PPO, the Head of the Independent Safety Office (TEC-QI) and the Head of the Quality Department (TEC-Q).

For missions, where the category is not clearly defined by the COSPAR Policy on Planetary Protection, e.g. Category II*, the PPO can organise consultations with the ESA PPAP and the COSPAR Planetary Protection Panel to receive multidisciplinary scientific advice for the categorization and Planetary Protection requirements.</td><td>The PPO</td></tr>
<tr><td>Planetary Protection Plan</td><td rowspan="8">N/A</td><td rowspan="6">The PPO</td><td colspan="3" rowspan="6">The PPO, the Head of the Independent Safety Office (TEC-QI) and the Head of the Quality Department (TEC-Q).</td><td rowspan="6">The PPO</td></tr>
<tr><td>Planetary Protection Implementation Plan</td></tr>
<tr><td>Pre-Launch Planetary Protection Report</td></tr>
<tr><td>Post-Launch Planetary Protection Report</td></tr>
<tr><td>Extended Mission Planetary Protection Report</td></tr>
<tr><td>End-of-Mission Planetary Protection Report</td></tr>
<tr><td>Nonconformance Report</td><td colspan="5">The PPO</td></tr>
<tr><td>Request for Waiver/Request for Deviation</td><td>The PPO</td><td colspan="3">The PPO, the Head of the Independent Safety Office (TEC-QI) and the Head of the Quality Department (TEC-Q).</td><td>The PPO</td></tr>
</table>

4.4.2.4 Head of the Independent Safety Office (TEC-QI)

The Head of the Independent Safety Office has the following responsibilities:

- Reviewing ESA missions' compliance with the ESA PP Policy.
- Approving ESA missions' compliance with the ESA PP Policy.
- Reviewing ESA restricted Earth return missions' compliance with the ESA PP Policy prior to the segments of the return phase.
- Chairing the ESA (Planetary Protection Advisory Panel (PPAP).
- Supporting the resolution of Planetary Protection relevant anomalies.

4.4.2.5 Planetary Protection Officer (PPO)

The **Planetary Protection Officer** has the following responsibilities:

- Reviewing ESA missions' compliance with the ESA PP Policy.
- Approving ESA missions' compliance with the ESA PP Policy (see Table 4-1).
- Fulfilling PP related obligations of relevant ESA external authorities.
- Reviewing ESA restricted Earth return missions' compliance with the ESA PP Policy prior to the segments of the return phase.
- Coordinating and cooperating with international partners.
- Representing ESA in relevant international bodies, in particular the COSPAR Planetary Protection Panel.
- Reporting to COSPAR about mission compliance with the COSPAR Policy on Planetary Protection.
- Ensuring independent verification assays on flight hardware and controlled environments, including launch sites to be conducted in the course of projects and especially at the final close-out of the flight hardware prior to launch.
- Reporting/advising the ESA Council on general and specific issues related to the ESA PP Policy and its associated requirements.
- Installation of the ESA PPAP and proposal of members to the Head of the Quality Department (TEC-Q).
- Being the executive secretary of the ESA PPAP.
- Supporting the development and maintenance of relevant policies, requirements and standards.
- Collecting initiatives and establish the Agency's R&D plan of activities in the subject of Planetary Protection, ensuring the management and distribution of these activities to the relevant Preas of expertise in the Agency.
- Supporting the resolution of Planetary Protection relevant anomalies.

4.4.2.6 ESA Planetary Protection Advisory Panel (PPAP)

The **ESA Planetary Protection Advisory Panel** (PPAP) has the task to advice the PPO and make recommendations on:

- The formulation and updating of the Planetary Protection Policy and requirements.
- The identification and prioritization of standards & procedures for implementing Planetary Protection requirements.
- The identification and prioritization of mid- and long-term research and technical development needs in the field of Planetary Protection.
- The Planetary Protection categorization and pre-launch certification of spaceflight missions.
- Spaceflight missions' compliance prior to the segments of the return phase of restricted Earth return missions.
- The risk resulting from the release of unsterilized particles during restricted Earth return missions.

The ESA PPAP starts its work at the request of the PPO. The Panel members are ESA internal and external subject matter experts (when applicable) of various disciplines from within ESA Member States. The members are appointed by the Head of the Quality Department (TEC-Q), based on recommendation from the PPO.

The Panel can have different member compositions, to make sure the right subject matter experts are present in the Panel for the specific tasks to be worked.

The PPO is the Executive Secretary of the ESA PPAP.

The Head of the Independent Safety Office (TEC-QI) is the Chair of the ESA PPAP.

For restricted Earth return missions, the ESA PPAP is called **Planetary Protection Re-Entry Safety Panel** (PPRSP).

4.4.2.7 Head of the Quality Department (TEC-Q)

The following responsibilities are with the H/TEC-Q:

- Approving ESA missions compliance with the ESA PP Policy.
- Appointing members of the ESA PPAP based on recommendation from the PPO.

4.5 Planetary Protection categorisation

4.5.1 Overview

Solar system bodies do not have the same level of sensitivity to contamination. In Planetary Protection there are five categories based on target body (i.e. Moon, Mars, Jupiter, etc.) and mission type (i.e. lander, orbiter, fly-by) combinations. A given category reflect the scientific interest and the concern that:

1. Terrestrial contamination can compromise future investigations;
2. Unsterilised extraterrestrial material brought by returning spacecrafts can cause detrimental effects to Earth environment and public health.

Categorisation defines a specific mission's biological contamination risk in a process which takes into account target body, target bodies encountered, hardware and operations.

Based on the contamination risk defined through the categorization process a range of technical requirements can be levied on projects, including:

- Cleanroom assembly.
- Hardware cleanliness.
- Trajectory bias.
- Inadvertent impact avoidance.
- Organic inventory.
- Sample containment.

Assignment of categories for specific mission and body combinations is determined by the best multidisciplinary scientific advice and it is a risk-informed decision-making process involving scientific consensus. That is to assess the risk of space activities introducing harmful contamination during the period of exploration or the possibility that extraterrestrial materials returning to Earth can have adverse impacts on the biosphere (see Figure 4-2).

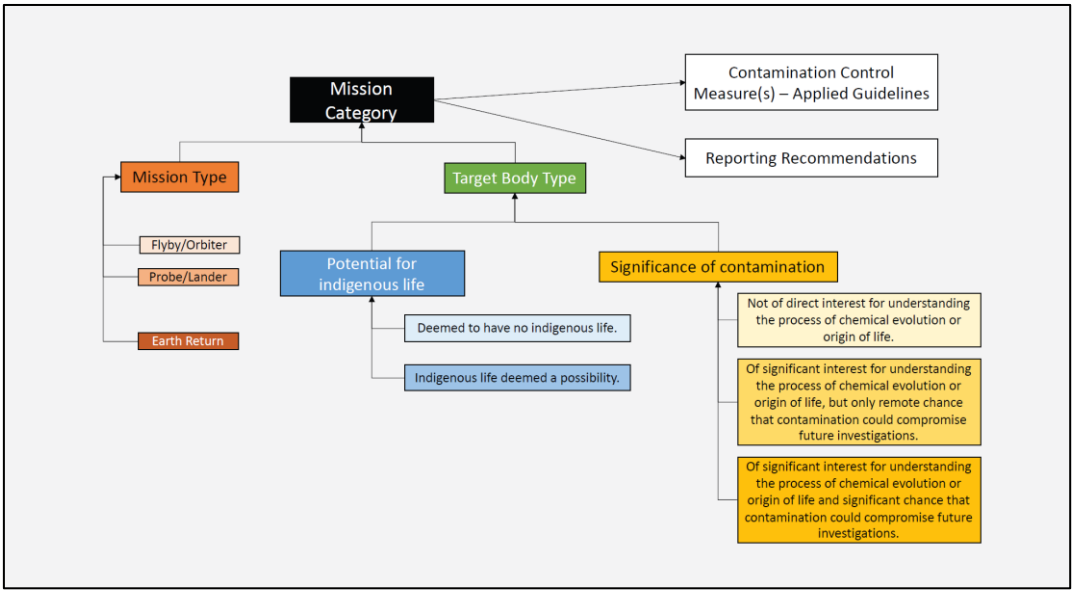


Figure 4-2: Process overview to establish a Planetary Protection categorisation and resulting guidelines for implementation and documentation definition (from COSPAR PP Policy, 2026)

From the COSPAR PP policy (2026), the type of mission and the target body type are the key parameters to establish a mission category. The type of mission includes flyby and orbiters where there is less likelihood for Planetary Protection related scientific investigation becoming compromised compared to a probe and a lander coming into direct contact with the target body. The target body type is evaluated on the potential for containing indigenous life and the significance for scientific investigation being compromised. Planetary Protection requirements can then be identified through the categorization process.

Several facors needs considerations during the categorization process:

- Target body(s),
- Main characteristic of the trajectory, including flybys or gravity assist maneuvres,
- Mission type (orbiter, lander),
- Target body(s) encounter(s) when going to final mission destination,

- End of mission plans for hardware, i.e. shutdown in place, dispositions, transfer to new location, including considerations on unsuccessfull disposal maneuver and off-nominal scenarios.

Current Planetary Protection categories are summarised in [RD2] and in Table 4-2.

Table 4-2: Planetary protecion categories as indicated in the COSPAR PP policy, 2024. (Please always refer to the latest version of the policy for the categorisation process)

Category	Mission type	Target Body
I	Flyby[1], Orbiter, Probe, Lander	Undifferentiated, metamorphosed asteroids; Io; others to-be-defined
II	Flyby, Orbiter, Probe, Lander	Venus[2]; Moon (Cat. II, IIa & IIb); Comets; Carbonaceous Chondrite Asteroids; Jupiter; Saturn; Uranus; Neptune; Icy Worlds[3]; Kuiper-belt objects that are not classified as Icy Worlds
III	Flyby, Orbiter	Mars; Icy Worlds[3]
IV	Probe, Landers	Mars (Cat. IVa, IVb, & IVc); Icy Worlds[3]
V	Unrestricted Earth Return	Venus; Moon; Small Solar System Bodies and Icy Worlds without an answer of "no" or "uncertain" to all 6 questions in section 6.5.2 of the COSPAR PP Policy 2026

[1] "Flybys" include gravity assist manoeuvres.

[2] For the categorization of Venus, see Zorzano-Meier et al. [2023].

[3] By default, missions to Icy Worlds are considered either Cat. III (Orbiter) or Cat. IV (Lander). Assignment of these missions to category II must be supported by an analysis that determines the probability of introducing any component of the spacecraft into >LLT environments that may exist beneath their surfaces within the Period of Biological Exploration (PBE) of 1000 years.

4.5.2 Categorisation process at ESA

The Planetary Protection categorisation is a multi-step and multidisciplinary process. The following **3 Steps**, summarised in Table 4-3, can be followed.

NOTE Only ESA missions which are not intending to leave Earth orbit are exempt from this process.

Table 4-3: ESA Categorisation process steps (R=Responsible; S=Supporting; I=Informed),

Step	What	Who
1	**The Project generates the PP category (and associated requirements) for the mission.** a. The Project Representatives (e.g. Study Manager, Project Manager) create the PP category for the mission by tailoring the ECSS-U-ST-20 and COSPAR PP policy (latest issue). b. For missions with one or more partners, the Planetary Protection categorisation and compliance to Planetary Protection requirements is the responsibility of the lead partner (either agency or commercial entity). For non-ESA-led missions, the ESA project ensure that the lead partner (either agency or commercial entity) or service provider applies Planetary Protection categories (and requirements) that are consistent with the COSPAR Policy on Planetary Protection. This implies that the	Project representative (R) PPO (I)

Step	What	Who
	technical requirements adopted by the service provider can be different from the ECSS-U-ST-20C requirements adopted by ESA, but still a compliance with the COSPAR Policy on Planetary Protection can be demonstrated. c. The Project Representatives (e.g. Study Manager, Project Manager) submit the preliminary PP category to the PPO as early as possible, latest at the PRR. d. In case of a potential change in the applicable requirements, e.g. due to new scientific discoveries related to the target body or a change of the mission scope and extension, the ESA PP Process restarts. The PPO can inform the project if any change in the COSPAR Policy on Planetary Protection that can change the category and associated project requirements.	
2	**The PPO reviews the PP category (and requirements)** e. The PPO reviews the PP category and requirements document f. If deemed necessary by the PPO (e.g. for restricted Earth return missions), the PPO is consulted by the ESA Planetary Protection Advisory Panel (PPAP) and the COSPAR Planetary Protection Panel to support the review. **g.** If there are needed changes the PPO informs the project and the project iterates to **Step 1**.	**PPO (R)** ESA PPAP (S) Project Representatives (I)
3	**The PPAA approves the PP category (and requirements)**	**PPO (R)** **PPAA (R)** Project Representatives (I)

4.5.3 Example of categorisation assignements in Planetary Protection:

Example 1 – Mission to asteroids

A mission is planned to target an asteroid, which is a **Category I** target. In doing so, it performs a flyby on Mars, which is **Category III**. The mission is then assigned a Planetary Protection **Category III** due to the Mars flyby.

Example 2 – Mission to Icy worlds

A mission is set to explore Jupiter's Moons Europa and Callisto with an orbiter; fly-bys are also planned to Ganymede, where the spacecraft can also be dispositioned at end of mission.

Orbiters around Europa are designated as missions **Category III**, to minimise probability of contaminating subsurface oceans to 10^{-4} ; missions to Callisto are Category II.

Attention needs to be given to the end-of-mission scenario, as the spacecraft can be dispositioned to Ganymede. As such, for Ganymede, an analysis can be done to support a "remote" potential to contaminate areas where T > -28 °C that can exist beneath the surface. This implies making use of international scientific consensous to address the existence of such environments, the possibility to access them (in nominal and **off-nominal** scenarios), and evaluate the probability of introducing a single

viable terrestrial organism which is able to proliferate. In case areas with T > -28°C are not accessed or the probability of contamination can be demonstrated to be < 1 x 10^{-4} , the specific portion of the mission to Ganymede can be Category II; on the contrary, Category III can also be assigned for Ganymede, with potential ramifications to the final decision to actually disposition a spacecraft on Ganymede.

The overall mission category shoud be **Category III**, due to Europa flybys.

Example 3 – Landing mission to Mars

A mission plans to land on Mars with a rover, which is equipped with analytical payloads (i.e. Gas Chromatographer, Mass Spectrometer) designed to look for extant or extinct life on the Red planet.

The mission category for landers to Mars is **IV**. However, it is improtant to ascertain the sub-category of such a mission, as associated requirements change considerably. As such, an study can be conducted to verify rover accessing to Mars special regions. This can be achieved either with vertical or horizontal mobility; nomincal and **off-nominal scenarios** need also to be considered.

Such a study can be based on scientific consensous and latest scientific evidence, including an assessment of the extent to which the tempearture and water activity values specified for Mars special regions are separated in time. It is also important to include the case of **induced special regions**, which can be created for example by radioisotope heat sources targeting areas with surface or subsurface water ice. Planned 3-sigma pre-launch landing elipses can be evaluated and documented on a case-by-case basis as part of the landing site selection process. That will determine whether the mission can land or come within contamination range of Mars special regions parameters or impinge on already described features that can be treated as Mars special regions. Such evaluation needs to be updated during the course of the mission, whenever new evidence indicates that the landing elipse and the operational environment contains (or are in contamination range of areas or volumes) parameters as defined for Mars special regions.

If the analysis can demonstrate that special regions won't be accessed, the mission can be categorised aas **Category IVb**; in case Mars special regions are accessed, then mission can be assigned a **Category IVc**.

Example 4 – Sample return mission

A mission plans to return samples to Earth from an asteroid; in doing so, a flyby to Mars is planned. The mission category can then be defined by splitting the outbound and inbound phases.

For forward contamination (outbound), the mission is **Category III**, due to a flyby on Mars. For backward contamination (inbound), the mission falls within **Category V**. At this point, an analysis needs to be performed to ascertain the subcategory, "unrestricted" or "restricted" Earth return. It is important to consider the following **six** points in the assessment and based on latest scientific evidence:

1. Assess whether or not liquid water was never present in or on target body.
2. Assess whether or not metabolically useful energy was never present.
3. Assess whether or not organic matter or CO^2 or carbonates and an appropriate source of reducing equivalents in or on the target body was never sufficient to support life.
4. Assess whether or not, subsequently to disappearance of liquid water, the target body has been subject to temperature > d 160° C.
5. Assess whether or not there is or was sufficient radiation for sterilisation of terrestrial life forms.
6. Assess whether or not there has been a natural influx to Earth, via meteorites, or material equivalent to a sample returned from the target body.

It is expected for an asteroid that the assessment for all of the 6 points above leads to **Category V** "unrestricted". To be noted that, if one or more of the points above is uncertain or cannot exclude presence of ingredients for life or natural sterilisation at target body, then **Category V** "restricted" Earth return is assumed

Example 5 – Mission to the Earth's Moon

A series of missions are planned to land on the Moon and deliver payloads, infrastructures for scientific operations, a rover, astronaut support cargo, and power stations. The mission is set to land on the south pole of the Moon.

The mission can be given a **Category IIb**, which is for landed missions going to a Permanently Shadowed Region (PSRs) and the Lunar poles.

NOTE Within the lanuguage used in the COSPAR PP policy for **Category IIb** missions, it is highlighted the importance of latituted south 79 °S and north 86 °N. This does not mean that only those latutudes are taken into account. The whole Lunar poles and PSRs are to be considered in the categorisation process.

5 Planetary Protection requirements verification

5.1 Overview

In order for ESA to comply with the overall principles and guidelines listed in the ESA PP policy, ESA Missions shall comply with the ECSS-U-ST-20 "Planetary Protection Requirements " [RD3].

For each requirement contained in [RD3], the structure of the sections of ESSB-HB-U-006 Handbook is summarized in Table 5-1.

Table 5-1: Structure of the sections of ESSB-HB-U-006 Handbook

Section Number	Section Title	Content
5.Y	Requirement	Text of the requirement
5.Y.1	Rationale for the Requirement	Background information and justification for the adoption of the requirement
5.Y.2	Methods to Assess Compliance	Guidelines on how to demonstrate project compliance with the requirement
5.Y.3	Mitigation Measures	Possible implementation approaches to comply with the requirement

5.2 Management requirements

5.2.1 Examples of management requirements

Typical management requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.1.

a. *The PPAA shall provide to the supplier Planetary Protection related obligations of relevant external authorities.*

b. *The supplier shall prepare a preliminary Planetary Protection Requirements document in conformance with the DRD in Annex A during the Phase A and no later than the PRR.*

c. *For missions that target or encounter multiple Solar system bodies, the preliminary Planetary Protection Requirements document shall include requirements for all the protected Solar system bodies.*

d. *The Planetary Protection Requirements document shall be subject to approval by the PPAA and released at the latest at SRR.*

e. *The delivery of hardware and services to a third-party mission with Planetary Protection constraints shall be subject to approval by the PPAA.*

f. *Impact of significant changes in the mission concept on the Planetary Protection requirements and implementation approach shall be assessed by the supplier and are subject to approval by the PPAA.*

g. *The PPAA, or its designated entity, shall conduct independent verification assays on flight hardware and controlled environments, including launch site, during the project at times and intervals planned and agreed with the supplier.*

5.2.2 Rationale for the requirement

These are typically specific project or mission 'management requirements', for e.g. related to the appointement of Planetary Protection Leads (PP-L) and their function; industrial prime contractor and launch service responsibilities; format and storage of Planetary Protection related data and analysis.

Management requirements aim to establish a process and a clear "chain of command", which is necessary to implement Planetary Protection programs throughout industrial organisations. This includes assignment of a project Planetary Protection lead, who can establish a link between project and the PPO at all stages of PP management. For the process to be followed and the definition of all PP functions, please refer to Section 4.4.

Consequence for not having a well-established management and process Planetary Protection in place can lead to difficulties in compliance with Planetary Protection requirements.

5.2.3 Methods to assess compliance

Management requirements for Planetary Protection are verified by Review. Audits and documents verification through Project Reviews are used to assess compliance to the PP management requirements. Verification is traced during VCBs (Verification Control Boards).

5.2.4 Mitigation measures

Possible mitigation measures to minimise risks associated to management requirement are:

- Defining job titles, PP roles and responsibilities at different levels, i.e. projects, space agencies, contractors. PP functions can be covered by already existing roles (i.e. system engineering) within the project teams.
- Providing a clear framework and work package in terms of tasks and responsibilities. Resources assignment needs to be budgeted and executed accordingly (e.g. by means of dedicated recruitment).
- Identifying a key contact person from the Project PP team, who acts as a link at all stages of PP management, setting in with the PPO, the contractors, and subcontractors, helping the easy circulation of relevant PP information and providing updates along the project.
- Considering Planetary Protection from early phases of a project, given the high impact that the Planetary Protection requirements can have on the overall project in terms of cost and time.
- Planning early and regular meetings with all PP related personnel involvedat 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.
- Defining KPIs (key performance indicators) to monitor the advancement of PP work packages and to provide early warnings in case of drifting with respect to the defined plan.

5.3 Cleanroom

5.3.1 Examples of cleanroom requirements

Typical cleanroom requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.2 and 5.4.:

Clause 5.2.1 Flight Hardware assembly

a. *Except as specified in 5.3.2.1d, all flight hardware subject to Planetary Protection constraints shall be assembled and maintained until and including launch in ISO class 8 cleanrooms "in operation", or better, as specified in ECSS-Q-ST-70-01.*

Clause 5.4.1 Bioburden-controlled environments

a. *Requirements from clause 5 of ECSS-Q-ST-70-58 shall be applied for bioburden-controlled environments.*

5.3.2 Rationale for the requirement

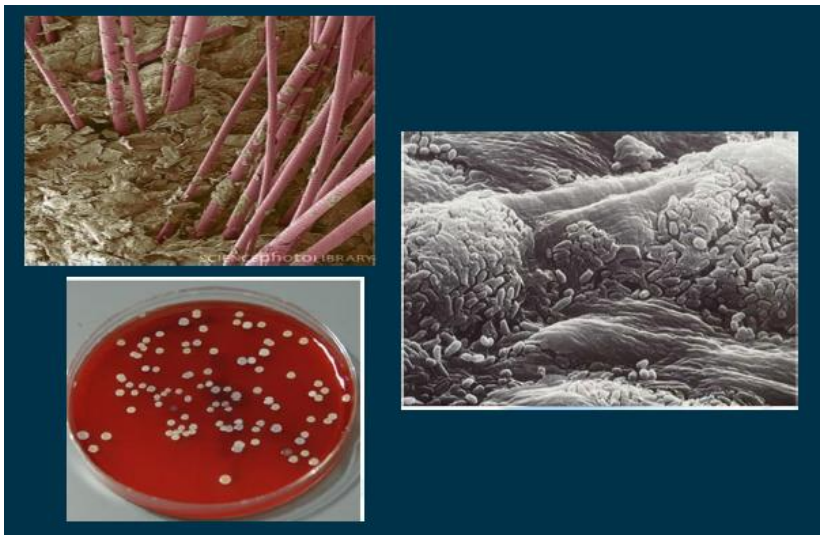
Cleanroom requirements aim to ensure that the flight hardware is kept under a controlled environment to limit the number of particles and excessive development of biological contamination. A cleanroom is an environment in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation and retention of particles inside the room, and in which other relevant parameters, e.g. temperature, humidity and pressure, are controlled as necessary. Given particle contamination is a typical carrier of bioburden, limiting the number, size and distribution of particle have beneficial effects on microbial contamination levels, as notionally showed in Hairs are a carrier for biological contamination, as it can be seen in the picture at the bottom, showing develop colonies forming units (CFUs) – white spots after locating an hair in the agar medium.

Figure 5-1. As an example of particulate contaminants to be passengers for biological contamination, the Hairs are a carrier for biological contamination, as it can be seen in the picture at the bottom, showing develop colonies forming units (CFUs) – white spots after locating an hair in the agar medium.

Figure 5-1 shows healthy human hair follicles at different magnifications and under SEM – Scanning Electronic Microscope. Microbial colonies can be clearly seen (right picture in Hairs are a carrier for biological contamination, as it can be seen in the picture at the bottom, showing develop colonies forming units (CFUs) – white spots after locating an hair in the agar medium.

Figure 5-1) on a human hair follicle. A hair is then taken and placed on a (blood) agar plates; after incubation, development of numerous colonies forming units (CFU) can be seen. These are the white "spots" seen in the bottom picture in Hairs are a carrier for biological contamination, as it can be seen in the picture at the bottom, showing develop colonies forming units (CFUs) – white spots after locating an hair in the agar medium.

Figure 5-1.



Hairs are a carrier for biological contamination, as it can be seen in the picture at the bottom, showing develop colonies forming units (CFUs) – white spots after locating an hair in the agar medium.

Figure 5-1: Human hair follicles shown at different magnifications under SEM – Scanning Electronic Microscope

A cleanroom "in operation" means that the installation of the cleanroom is complete, the HVAC (Heating Ventilation and Air Conditioning) system is fully operational, equipment installed and functioning in nominal operating mode, with personnel (maximum number) working inside the room in operational conditions.

A cleanroom in operation differs from one "at rest". The latter is the condition whereby the installation of all the utilities is complete including any functioning HVAC, with the main manufacturing equipment installed as specified, but not operating and without personnel present in the room.

A bioburden control cleanroom is a room where microbiological contamination cleaning and monitoring procedures are in place (in addition to particle airborne) to meet given Planetary Protection constraints.

5.3.3 Methods to assess compliance

The methods to verify these requirements are by reviewing relevant documentation, i.e. cleanroom commissioning and reports, cleanliness and Planetary Protection plans; and inspecting facilities to verify that all requirements within ECSS-Q-ST-70-01 and ECSS-Q-ST-70-58 are met.

Any airborne controlled environment is classified according to ISO 14644‑1:1999. Classification goes from 1 (most restrictive) to 9 (less restrictive).

In Figure 5-2 is shown the number of particles per cubic meter as a function of the diameter, from 0,1 μm to 5 μm (derived from ISO 14644‑1:1999), including ISO 8 classification information.

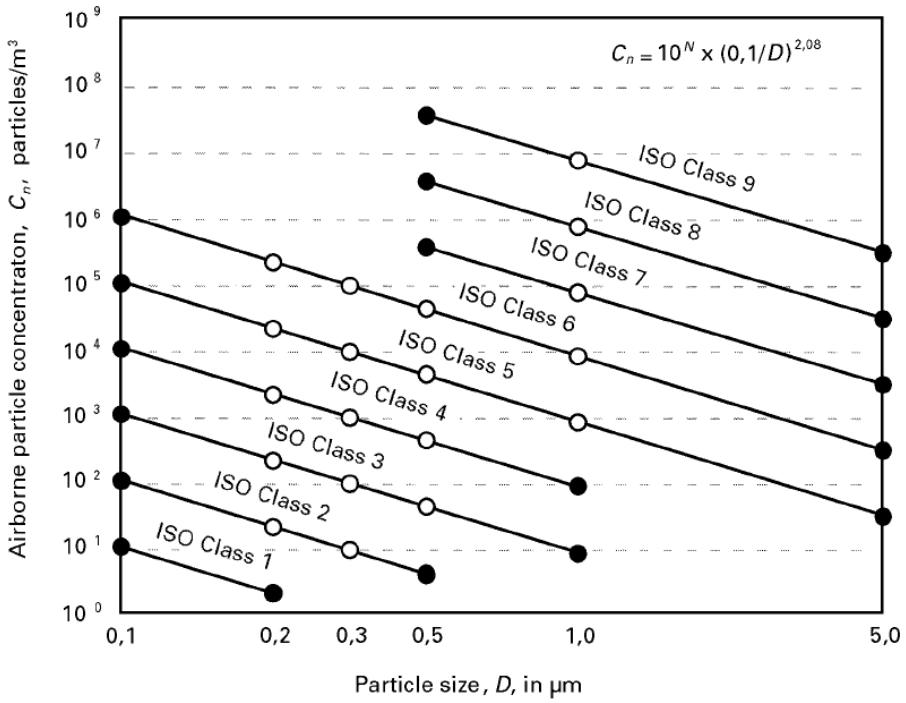


Figure 5-2: Graphical representation of ISO-class concentration limits for a given ISO class (extract from ECSS-Q-ST-70-01)

Table 5-2 shows selected airborne particulate cleanliness classes and the corresponding particle concentrations for particles equal to and larger than the considered sizes shown. The maximum permitted concentration of particles, Cn, for each considered particle size, D, is determined from the equation:

Cn=10N·(0,1/D)2,08

where:

- **Cn** is the maximum permitted concentration (in particles per cubic metre of air) of airborne particles that are equal to or is rounded to the nearest whole number, using no more than three significant figures;
- **N** is the ISO classification number, which does not exceed a value of 9. Intermediate ISO classification numbers can be specified, with 0,1 the smallest permitted increment of N;
- **D** is the considered particle size, in micrometres;
- **0,1** is a constant, with a dimension of micrometres.

From the particle point of view, the number of 5 µm particles per given volume of air is much more critical than the number of smaller particles, since the fallout is mainly determined by particles of 5 µm or larger. The cleanliness level of a cleanroom can only be selected when the specified obscuration factors for critical spacecraft surfaces are known. The particle size 5 µm is often used as a criterion, because for optical surfaces particles larger than 5 µm are critical, whereas for bearings and gears, particles in the range 10 µm to 40 µm are more harmful.

Table 5-2 : Airborne particulate cleanliness classes

ISO classification number (*N*)	Maximum concentration limits (particles/m³ of air) for particles equal to and larger than the considered sizes shown below (concentration limits are calculated in accordance with equation (1) in 3.2)					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	10 000	2 370	1 020	352	83	
ISO Class 5	100 000	23 700	10 200	3 520	832	29
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7				352 000	83 200	2 930
ISO Class 8				3 520 000	832 000	29 300
ISO Class 9				35 200 000	8 320 000	293 000
NOTE Uncertainties related to the measurement process require that concentration data with no more than three significant figures be used in determining the classification level						

The activities related to requirements for bioburden control cleanrooms, including specifications, procedures and reports are described in Figure 5-3, extract from ECSS-Q-ST-70-01.

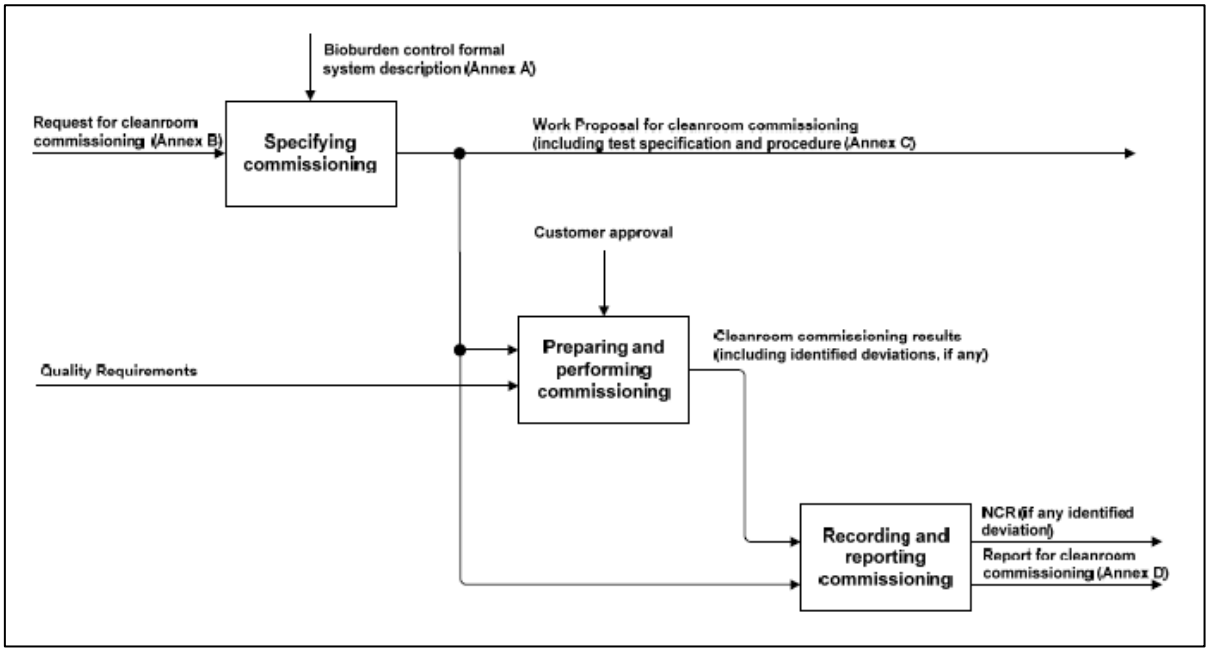


Figure 5-3: Bioburden control in cleanrooms: examination process overview. (The Figure 5-3 is taken from ECSS-Q-ST-70-58. The Annexes mentioned in the Figure 5-3 refers to ECSS-Q-ST-70-58)

As stated in ECSS-Q-ST-70-58, bioburden-controlled cleanroom includes a system (as a minimum) in place that:

- Establishes microbial thresholds to ensure control, including alert and action level as needed.
- Describes how the monitoring scheme (including frequency) is performed.
- Describes the validation procedures verify that the cleanroom is working properly.
- Establishes corrective actions in conformance with specific requirement.

- Assesses the risk associated with bioburden control via FMECA.
- Describes the training procedures and establishes access constraints.
- Maintains documentation, as electronic logbooks or process implementation data.
- Performs periodic reviews and audits.
- Maintains status of equipment.
- Establishes a training programme for personnel.
- Describes sampling plans, including at least:
  - The cleanliness level of the cleanroom to be applied and the appropriate degree of bioburden control for the activity being conducted.
  - The sampling location(s) identified including "risk zone; i.e.; the work area immediately adjacent to any contamination-critical hardware and the work activities and personnel associated with this location.
  - The minimum number of samples to be taken in order to ensure representative results.

    NOTE This number is dependent on the sample size and volume. For small sample sizes, representativeness of the results cannot be guaranteed if this number is not significantly increased.
  - Frequency of initial monitoring sampling, established in conformance with requirement as applicable.
  - Frequency of normal routine sampling, established in conformance with requirement as applicable.
  - Frequency of sampling for specific cases, including specific, non-nominal cases, for e.g. Suspected contamination events, Action levels are exceeded, Alert levels are exceeded (consecutively), A change in the biodiversity, After prolonged shut-down of activities, After any significant maintenance work has been undertaken on the cleanroom, Maintenance on ventilation system, changes to furnishings or fittings, introduction of significant quantities of new hardware or equipment, After changes to the process that affect the cleanroom environment, After non-routine changes to the cleaning or disinfection procedures, After any incidence of non-conformance to cleanroom operational specifications.

    NOTE Breaches of personnel dress and conduct code; out of specification airborne particulate levels, out of specification temperature or humidity levels, exceeding maximum number of personnel, or introduction of non-sterile items or hardware can also be considered as exceptions and sampling foreseen.

5.3.4 Mitigation measures

Design phase

The following aspects can be considered for ISO cleanrooms during the design phase:

- Cleanroom shell, floors, walls and ceiling need to be selected to be low shedding and the finish readily cleanable.
- Covering floors needs to be made of one piece or, if this is not feasible, a minimum number of joints.
- The floor needs to be resistant to withstand wear.

- The room needs to be designed such that there are interlocks, i.e. only one door can be opened at one time, except in case of emergency.
- Entrances need to be equipped with an air lock, to minimise contamination and help maintaining pressurisation of the area.
- Changing rooms needs to be equipped for the changing of clothes and the storage of clothing, personal belongings and cleaning equipment.

For bioburden-controlled cleanroom, the following aspects needs consideration, in addition to the points highlighted above for standard ISO classified cleanrooms:

- Vetting and control of all materials of construction, particularly organics, with controls limits on volatile organic compounds (VOCs) of build materials.
- Microbial antiproliferation technologies, i.e. silver ion, can be used for the floor, to help minimising the spread of biological contamination.
- The material choice for floors and walls needs to consider the use of aggressive chemical(s) used for cleaning operations in bioburden-controlled cleanrooms. Cleaning agents and chemicals can be multiple and rotated weekly to avoid microbial adaptation to one single chemical product.
- An air-shower needs to be foreseen in cleanroom design, and it can be positioned before entering main biologically controlled areas. Air-showers serve the main scope of removing particles cumulated during the dressing procedure in changing rooms, see Figure 5-4.
- Biologically controlled areas cannot be accessed directly from the outside world. Access to the bioburden-controlled environment can be done through successive layers of controlled environments (see Figure 5-5), e.g., from uncontrolled environment through a standard ISO 8 environment, to a standard ISO 7 environment before entering the bioburden-controlled ISO 7, environment. Different environments can be accommodated in a single cleanroom.

Multiple nozzles on the shower walls push HEPA filtered air toward the operator. This process generally takes 30 - 45 seconds: while clean air is supplied, the perators make sure that all the body gets hitted by the jettisoned air, e.g. by turning around continously and raising arms and feet for the whole duration of the air shower.

Figure 5-4: Personnel inside the air shower before entering a bioburden-controlled cleanroom (credit: Airbus Stevenage UK)

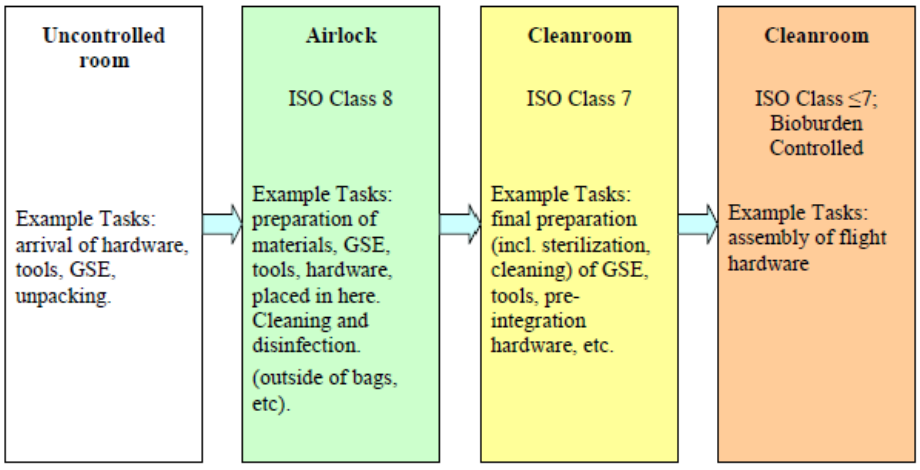


Figure 5-5: Cleanroom environment schematic (extract from ECSS-Q-ST-70-58)

Air supply

- Air supply and filtration equipment need to have the capacity to filter all new and recirculated air entering the room to guarantee the defined ISO class.
- Air conditioning equipment for prefiltering (particular and molecular), cooling, heating, humidification and dehumidification of the cleanroom air supply needs to be supplied to guarantee the environmental conditions.
- In laminar flow cleanrooms, the air flow velocity through the cross-section of the room needs to be maintained at 27 m/min with a uniformity within ± 20 % throughout the undisturbed room.
- Airflow patterns need to be uniform with minimum turbulence.

Filters

- In laminar flow cleanrooms, HEPA filters need to cover either one entire wall or the entire ceiling, except when diffusion ceiling or wall systems are used or when built-in benches are included in the incoming air end of the room.
- Monitoring needs to be done and any work with highly sensitive equipment cannot be performed before the defined ISO class for the hardware has been reached. as specified in the cleanliness plan for the following situations:

    1. After the installation of new filters.
    2. After "at rest " period.
    3. After "stand by" period.

Due to the transitory pressure gradients, contamination previously trapped by HEPA filters, together with a reduction in the operating life of the filters themselves can be released.

a. The air flow inside cleanrooms and independent HEPA filtering systems needs to be maintained during "at-rest" periods, except for the maintenance operations.

    NOTE 1 For example, during filters replacement.

    NOTE 2 Independent HEPA filtering systems can be like those used for the laminar flow tents and benches.

    NOTE 3 This is to avoid the risk of redistribution of particles at restart of the flow.

    NOTE 4 Exception can be made for independent HEPA filtering systems that can work with a reduced air flow rate during stand-by periods.

b. In cases where a uniform and controlled molecular environment is needed, the filtering system needs to be equipped with additional charcoal filters positioned before the HEPA filters.

When charcoals filters are used, the initial charge needs to be assessed on installation and analysed regularly.

NOTE It can be useful to evaluate the charge in contaminants of the filtering system which can release its charge in contaminants trapped. That is in order to be able to monitor the evolution and when a failure occurs.

Running a bioburden-controlled facility

A bioburden-controlled cleanroom is divided into specific areas. Each of those compartments is operated with ad-hoc requirements and procedures. Colour coding nicknames can be used to facilitate distinctions among bioburden-controlled and normal ISO standard rooms.

For example, the "white area" can nominally operate as an ISO 8 (or better) – "highly controlled" (HC) facility. This is where the main Assembly and Integration of biologically controlled hardware (e.g. COSPAR **Category IV** – landers to Mars) are performed. The classification "HC" means that in the ISO cleanroom bioburden contamination is strictly controlled, in addition to particulate (and molecular) contamination.

A "grey" area can be a preparation room, where for e.g. tools, ground support equipment is cleaned, sub-assemblies are made, or where sterilisation is performed prior to accessing "white" areas. A grey area generally operates as an ISO 8 or better, and it does not require to be HC – highly controlled, in terms of bioburden.

A "black area" can be an air lock or a changing room. The latter is notoriously the dirtiest area in a cleanroom, as personnel can access from a lobby or directly from the outside world, i.e. with street cloths. Great attention needs to be paid during the dressing procedure to make sure contamination is not transferred in higher cleanliness areas (grey and white). Most contamination brought inside cleanrooms is coming from people, as shown in Figure 5-6.

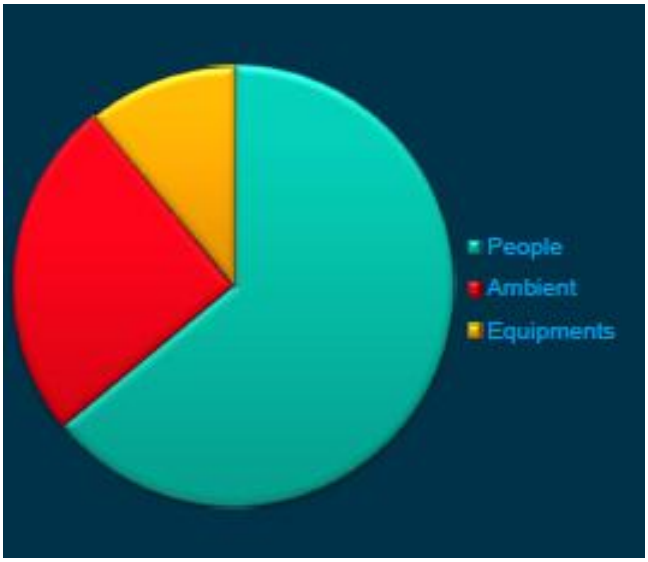


Figure 5-6: Notional representation of contamination sources in cleanrooms. To be noted, over 70 % are coming from personnel

Cleanroom clothing can be different for each of the areas identified in a bioburden-controlled cleanroom. A white area (i.e. highly biologically controlled) can require the use of sterile garments. These include sterile overall, sterile hoods, sterile (over) boots, sterile masks, sterile gloves (two pairs). Street cloths cannot be allowed in white areas, so the use of undergarments (pyjamas) is recommended. These do not need to be sterile, but they can be only washed or cleaned.

Biologically controlled areas garments can be worn for one shift maximum and then send for washing and cleaning. In cases where a white area is accessed more than one per day, a new, fresh set of sterile garments can be worn.

For non-biologically controlled areas, i.e. lobbies, airlocks, preparation, non-sterile garments can be worn. Their frequency for use cannot go over one week though.

Table 5-3 suggests typical clothing that can be worn in the different areas of biologically controlled environments.

Table 5-3 : Typical cleanroom clothing vs cleanroom area

Clothing type	Black areas, i.e. Lobby/Changing rooms	Grey areas, i.e. Airlocks	White areas, i.e. biologically controlled
Coverall	Nonsterile / disposable	Nonsterile / disposable	Sterile overall
Hoods	Nonsterile/ disposable	Nonsterile/ disposable	Sterile hood
Boots / Over boots	Nonsterile/ disposable	Nonsterile/ disposable	Sterile boots or overbooks
Face masks	-	Sterile or non-sterile	Sterile
Under garments	-	Street cloths	Sterile
Gloves	Single layer, they can be nonsterile, standard ISO 8	Single layer, they can be nonsterile, standard ISO 8	Double layer, commonly used Kimtech G3 (colour grey)

Figure 5-7 shows how personnel look like when working in biologically controlled cleanrooms.

Figure 5-7: Airbus UK (left) and TASI Turin (right) personnel appraisal in biologically controlled cleanrooms during ExoMars rover assembly, integration and launch operations

The monitoring of highly controlled cleanrooms can be done using Annex 1 of the EU GMP – European Commission, Guidelines for good Manufacturing Practice for medical products for human and veterinary use. EU GMP standard provides the maximum limits for microbial contamination that can be used for each grade, see Table 5-4.

Table 5-4: Maximum permitted microbial contamination during qualification
(extract from EU GMP)

Grade	Air sample CFU/m^3	Settle plates (diameter 90 mm) CFU/4 hours [a]	Contact plates (diameter 55 mm) CFU/plate
A	No growth		
B	10	5	5
C	100	50	25
D	200	100	50

a) *Settle plates should be exposed for the duration of operations and changed as required after a maximum of 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media used* (extract from EU GMP).

Grade A: The critical zone for high-risk operations (e.g. aseptic processing line, handling ultra clean zones connections, requiring aseptic operation.

Grade B: For aseptic preparation and purging, this is the background cleanroom for **Grade A** (where it is not an isolator). Air pressure differences need to be continuously monitored.

Grade C and D: These are cleanrooms used for carrying out less critical stages in the manufacture as a background for isolators.

For Category IV landers to Mars, **Grade B** is usually recommended for all operations, except when UCZ – ultra clean zones are handled. In that case a **Grade A** can be considered

Settle plates need to be exposed for the duration of operations and changed as needed after a maximum of 4 hours. Exposure time is based on recovery studies and cannot allow desiccation of the media used.

An approximate correlation can be made between the grade of a highly controlled cleanroom and its ISO classification, as indicated in Table 5-5.

Table 5-5 : Approximate correlation between ISO airborne classification and bioburden grade. Grade A is approximately ISO5, Grade B between ISO5/7, Grade C between ISO7/8 and Grade D between ISO8 / non ISO defined (Extract from EU GMP)

Grade	Maximum limits for total particle ≥ 0.5 µm/m^3		Maximum limits for total particle ≥ 5 µm/m^3	
	at rest	in operation	at rest	in operation
A	3 520	3 520	Not specified [a]	Not specified [a]
B	3 520	352 000	Not specified [a]	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [b]	29 300	Not predetermined [b]

a. *Classification including 5 µ m particles may be considered where indicated by the CCS or historical trends*

b. *For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable* (extract from EU GMP) .

5.4 Probability of impact

5.4.1 Examples of probability of impact requirements

Typical probability of impact requirements for Planetary Protection can be found in ECSS-U-ST-20C clauses 5.2 and 5.3.

Clause 5.2.2 Probability of impact

a. *A probability of impact analysis on protected Solar system bodies shall be performed and a report delivered for customer and PPAA review.*

b. *The probability of impact analysis specified in 5.2.2a shall include:*

1. *Single and multiple pass analysis;*
2. *Hardware, software and operational reliability;*
3. *Meteoroid impacts and effects on spacecraft reliability;*

   (a) Meteoroid model (e.g., IMEM 1.1) and parameters (fluence, directionality, velocity, density and margins) as defined in the project applicable environmental specifications

   (b) Damage equations in IADC-WD-00-03 for the damage assessment
4. *Spacecraft state including location, and velocity vector;*
5. *Manoeuvre and planet and satellite ephemeris uncertainty;*
6. *Stochastic variability of the atmospheric density with the amplitude of the Solar cycle estimated for the mission and sun epoch.*

NOTE 1 Different meteoroid models are used depending on the mission profile; dedicated models are currently developed for the Jovian system.

NOTE 2 Requirements that need a probability of impact analysis to demonstrate compliance are described in 5.3.2.1d, 5.3.2.1e.1, and 5.3.3.1b.4.

Clause 5.3.2.1 Mars missions general requirements

d. *The probability of impact on Mars by any element not assembled and maintained in at least ISO class 8 conditions shall be ≤ 1x10-4 for the first 50 years after launch for nominal and off-nominal flight conditions.*

NOTE Examples are upper stages.

e. *One of the following conditions shall be met:*

1. *The probability of impact on Mars by any part of a spacecraft assembled and maintained in ISO class 8 conditions, or better, is ≤ 1x10-2 for the first 20 years after launch, and ≤ 5x10-2 for the time period from 20 to 50 years after launch for nominal and off-nominal flight conditions.*
2. *The total bioburden of the spacecraft on Mars, including surface, mated, and encapsulated bioburden, is ≤ 5x105 bacterial spores.*

NOTE This requirement is also applicable for fly-by and gravity assist manoeuvres.

5.4.2 Rationale for the requirement

Spacecraft and launch vehicle's upper stages used for interplanetary missions, or missions to the libration points, can be inserted into orbits that have a non-zero probability to return to the Earth or impact other celestial bodies. In the frame of Planetary Protection, the probability of these events happening is estimated as impact represents potential hazard of contamination.

Impact requirements can be met during the whole duration of the PBE – Period of biological exploration. The PBE is the time during which a Solar system body is explored for signs of the origin of life and the history of prebiotic chemistry based on current scientific understanding. This time has been set to 50 years for Mars mission.

To be noted that the probability of impact requirement becomes less restrictive when a spacecraft is assembled in an ISO 8 cleanroom (or better). Such probability goes from 1 % for the first 20 years, to 5 % from 20 - 50 years when flight hardware is assembled in ISO 8 conditions, to 0,1 % when it is not assembled in ISO 8 cleanroom conditions. That is due to the different level of bioburden estimated to be carried in spacecrafts and based on assembly done in different cleanroom conditions. It can be also noted that the probability of impact increases with time of reaching orbit around Mars. That takes into consideration in-situ sterilisation effects (the longer the time, the higher is the sterilisation due to space environmental conditions, e.g. desiccation, UV, cosmic radiation) and a potential degradation of stable Martian orbits over time.

In case project cannot demonstrate compliance with the probabilistic requirements indicated in the requirements, a full bioburden control can be applied to the spacecraft (see bioburden assessment).

5.4.3 Methods to assess compliance

Considering the long-term range of trajectory propagation analysis (i.e. more than 50 years), the trajectory evolution is studied considering uncertainties coming from different sources. In particular, the following considerations are fed within the analysis:

1. Fly-bys of a celestial body can significantly influence the trajectory of a spacecraft and act as a multiplier of small differences among similar trajectories; the consideration of multi-pass trajectories is intended to characterise such potential spread of outcomes.
2. Degraded scenarios, e.g. in case of failures or in case of inaccuracies in the orbit injection.
3. Impacts with meteoroids and space debris can cause failures and/or generate a cloud of fragments.
4. The uncertainty in the knowledge of the spacecraft state is evaluated considering relevant factors such as the dispersion of the launch vehicle injection (usually provided in the form of a covariance matrix), and the limit in the accuracy in the orbit determination process. In addition, the variability in the spacecraft parameters (e.g. cross-sectional area, reflectivity coefficient) can also be considered, e.g. with distribution between the minimum and maximum value, accounting for relevant space system configurations (e.g. Solar array fully deployed, partially deployed, not deployed).
5. The uncertainties in the implementation of manoeuvres (both in terms of magnitude and direction) and in the ephemerides of the celestial bodies, including, if relevant, the variability across the epoch of launch, injection, separation, or relevant manoeuvre, e.g. with uniform distribution across time interval or at discrete times, (e.g. in case of an extended launch window).
6. The effect of the atmosphere and its variability depending on the predicted Solar activity.

A statistical analysis is performed to compute the impact probability and account for the abovementioned sources of uncertainty. The trajectory propagation is performed considering at least:

- The gravitational influence of the Sun, the planets in the Solar System, and natural satellites when relevant.
- The effect of the Solar Radiation Pressure, at least with a cannonball model.
- Other perturbative forces, such as the non-uniform gravitational field of planets and moons, are included when relevant. A case-specific dedicated perturbation magnitude analysis is included to prove the soundness of the selected models.

A hierarchical approach for the different contributions to the dynamical models can be used, to consider the different effects only when statistically relevant in the scope of the overall analysis. That is providing sufficient validation of the proposed approach is included, either directly in the analysis or in the appended literature.

It is common to perform the trajectory propagation using an integration technique that allows for the control of the truncation error such as the Runge-Kutta and Adams-Bashforth numerical schemes.

For what concerns the ephemerides of the celestial bodies, the JPL SPICE toolkit provides an extensive library of binary kernel files containing the coefficients of Chebyshev polynomials fitted to the Cartesian positions and velocities of celestial bodies for the computation of the ephemeris. It also includes different functions for the computation and manipulation of ephemeris data.

Other ephemeris toolkits and data can be used, provided that sufficient validation against comparable JPL SPICE cases is available in the analysis itself or in the appended literature. The accuracy for the retrieval of position and velocities of the considered bodies needs matching with the JPL SPICE toolkit, within the confidence interval stipulated by the analysis.

Probability computations are usually performed using Monte Carlo techniques with a Cartesian formulation of orbital dynamics. When Monte Carlo simulations are performed, the minimum number of runs is set to ensure, with a minimum confidence level, that the solution, which is not known *a priori*, is convergent as the number of runs further increases.

In the case of Planetary Protection, the problem can be formulated with a binary criterion (i.e. impact/no-impact), a single Monte Carlo run can be considered as a binomial process with only two possible outcomes, generally labelled as "success" and "failure". The output of the Monte Carlo simulation is considered as a binomial variable $X \sim B(n,p)$, with n number of trials and p success probability for each trial. Applying an approximation to a normal distribution, the mean value is $\mu = np$ and the variance is $\sigma = np(1-p)$, and the confidence interval (Wald's confidence level) is:

$$\hat{p} - z_{1-\alpha/2}\sqrt{\frac{1}{n}\hat{p}(1-\hat{p})} \leq p \leq \hat{p} + z_{1-\frac{\alpha}{2}}\sqrt{\frac{1}{n}\hat{p}(1-\hat{p})} \qquad \text{[M-1]}$$

where $\hat{p}$ is the probability of success estimated from the statistical sample, $z_{1-\frac{\alpha}{2}}$ is the $\left(1-\frac{\alpha}{2}\right)$ quantile of a standard normal distribution, $\alpha = 1 - c$ is the error quantile, with c confidence level.

When the probability p tends to 1 or 0, a more adequate estimation of the confidence level (Wilson's confidence level) is used, considering that, for an observed value $\hat{p}$, there are two values of the mean p of a normal distributed variable that can put $\hat{p}$ at the limits of a confidence interval about p:

$$\frac{\hat{p} + \frac{z^2_{\frac{\alpha}{2}}}{2n} - z_{\frac{\alpha}{2}}\sqrt{\frac{\hat{p}(1-\hat{p})}{n} + \frac{z^2_{\frac{\alpha}{2}}}{4n^2}}}{1 + \frac{z^2_{\frac{\alpha}{2}}}{n}} \leq p \leq \frac{\hat{p} + \frac{z^2_{\frac{\alpha}{2}}}{2n} + z_{\frac{\alpha}{2}}\sqrt{\frac{\hat{p}(1-\hat{p})}{n} + \frac{z^2_{\frac{\alpha}{2}}}{4n^2}}}{1 + \frac{z^2_{\frac{\alpha}{2}}}{n}} \qquad \text{[M-2]}$$

[M-2] is not suitable for a low number of simulations ($n < 30$) since it an approximation for the discrete binominal distribution. For higher number of simulations ($n > 30$), an approximation for continuity can be used, (e.g. Yale's correction, which is demonstrated to be conservative for $n > 50$):

$$\hat{p} - \left(\frac{1}{2n} + z_{\frac{\alpha}{2}} \sqrt{\frac{\hat{p}(1-\hat{p})}{n}} \right) \leq p \leq \hat{p} + \left(\frac{1}{2n} + z_{\frac{\alpha}{2}} \sqrt{\frac{\hat{p}(1-\hat{p})}{n}} \right) \quad \text{[M-3]}$$

In the case where an explicit target for p exists, the expression of Wilson's confidence interval can be used, for example, to determine the minimum number of runs with no *failures* ($\hat{p} = 0$) needed to ensure that p is below the defined threshold, with the selected confidence interval.

Alternative formulations of the orbital dynamics can be used, provided that the resulting accuracy of the propagated trajectories is greater than or equal to the baseline Cartesian case. Other statistical approaches can be used, provided that the resulting impact probability estimate matches Monte-Carlo strategies within a greater than or equal to confidence level, for the same analysis. Sufficient validation of the selected technique needs to be available either as part of the analysis itself or in the appended literature.

Degraded scenarios are treated by considering the list of feared events and the corresponding probability. A functional Fault Tree can be identified to quantify the combined casualty risk of the nominal scenario and of off-nominal cases.

A FMECA [RD16], identifying the contributors to the off-nominal scenarios and their occurrences can be used as an input to verify this Fault Tree model. The sum of all weighted impact probabilities for all scenarios per mission is then the value to be compared to a threshold defined in specific requirements.

5.4.4 Mitigation measures

For any spacecraft not directed towards one of the protected Solar system bodies, execute a disposal manoeuvre such that to minimise the return probability to regions of interest, for the specified timeframe.

For launch vehicles, when possible, execute a disposal manoeuvre resulting in a controlled re-entry towards the Earth, or a disposal towards a stable heliocentric orbit.

The disposal of launch vehicles used for interplanetary injection needs to be considered too. Interplanetary injections are executed such that the collision probability of the disposed launch vehicle with bodies of interest is minimised. In case of potential returns to the Earth-Moon system, it is important to check that the disposal plan is defined in accordance with the applicable space debris mitigation requirements [RD12].

CUDAjectory's is the tool used by ESA to perform large Monte Carlo simulations, given its parallelisation capabilities. CUDAjectory computational power allows producing reliable and accurate collision probability estimates. A guide on how to configure and use CUDAjectory is shown in Annex A.3.

Html up-to-date files are also available online.

5.5 Probability of contamination

5.5.1 Examples of probability of contamination requirements

Typical probability of contamination requirements for Planetary Protection can be found in ECSS-U-ST-20C clauses 5.2 and 5.3.

Clause 5.2.3 Probability of contamination

a. *Except where numerical requirements are otherwise specified in this document, the probability of contaminating a Solar system body with viable terrestrial organisms shall be ≤ 1x10-3 over a period of 50 years after the arrival of the mission at the protected Solar system body.*

NOTE *Description how compliance to this requirement will be demonstrated needs to be described in the Planetary Protection Plan DRD in B.2.1a.3.(e).*

Clause 5.3.2.2.2 Requirements for Mars surface missions

e. *The evaluations specified in 5.3.2.2.2c and 5.3.2.2.2d shall be based on the latest scientific evidence and include an assessment of the extent to which the temperature and water activity values specified for Mars Special Regions are separated in time.*

Clause 5.3.3.1 Missions to Europa and Enceladus

a. *The probability of inadvertent contamination of a subsurface ocean by viable terrestrial organisms shall be ≤ 1 x 10-4 per mission.*

b. *The calculation of the probability specified in 5.3.3.1a of inadvertent contamination shall address the following factors, at a minimum:*

1. *Bioburden at launch;*
2. *Cruise survival for bioburden;*
3. *Bioburden survival in the respective radiation environment;*
4. *Probability of landing on Solar system body;*
5. *The mechanisms and timescales of transport to the subsurface;*
6. *Bioburden survival and proliferation before, during, and after subsurface transfer.*

NOTE *Methods of bioburden reduction reflect the type of environments found on Europa or Enceladus, focusing on terrestrial organisms most likely to survive on Europa or Enceladus, such with cold, desiccation and radiation tolerance.*

c. *When computing the calculation of requirement 5.3.3.1b the estimate for poorly known parameters shall be subject to approval by the customer and the PPAA.*

5.5.2 Rationale for the requirement

Limiting the probability of contamination is the preferred method to reach the overall Planetary Protection objective of protecting future search for life in outer space from harmful contamination (i.e. detrimental to understand the process of chemical evolution and origin of life in the Solar system).

A probabilistic approach to Planetary Protection includes the use of mathematical models to estimate the likelyhood that microbes from spacecrafts can contaminate a potentially habitable environment, such as the subsurface of an Icy Worlds or special regions on Mars. In doing so, several aspects are typically considered in a multidisciplinary activity which involves: microbiology (to estimate bioburden at launch and its survival during journey in space and at target body), engineering and mission analysis (to spacecraft design and reliability, calculate trajectories and impact probability), planetary science (to understand transport mechanisms that can cause harmful contamination events), statistics and mathematics (to generate tailored comprhensive mathematical models and integrate data & assumptions).

This approach relies on probability risk assessment, which combines qualitative and quantitative data to analyse risks in complex systems. In Planetary Protection, it supports risk-informed decision-making by evaluating how effectively a mission prevents forward contamination and by integrating modern scientific and statistical methods. A list of objectives helping the built of risk-inform decision frameworks is shown in Figure 5-8, from NASA HDBK, reference SP-20240016475.

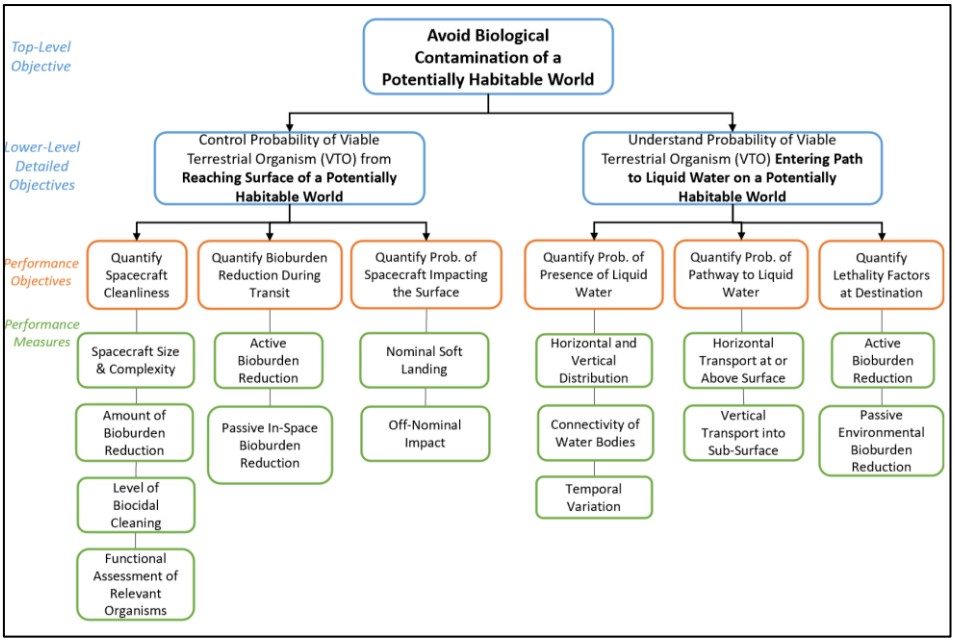


Figure 5-8: Risk informed decision-making objectives for harmful contamination, extract from NASA HDBK , SP-20240016475 Version 1.0

Performance metrics for Planetary Protection are based on current scientific understanding and international consensus, and each objective helps determine the probability of contamination. However, several unknowns need to be addressed. These include the identification of contamination sources, understanding their behaviour, assessing microbial responses to sterilisation (including in-situ, e.g. in outer space), predicting spacecraft trajectories, knowing environmental and material exchange processes on target bodies, estimating conditions during transit and at the destination, and building probability models. A crucial factor is to accurately determine how many viable microbes are present from different contamination sources throughout the mission. Modern models still require to be fed with credible experimental data. Without scientific knowledge of (currently poorly known) parameters such as bioburden at launch, their survivability during space journey and how contamination is transported at target body, stricter engineering procedures are necessary, as notionally shown in Figure 5-9.

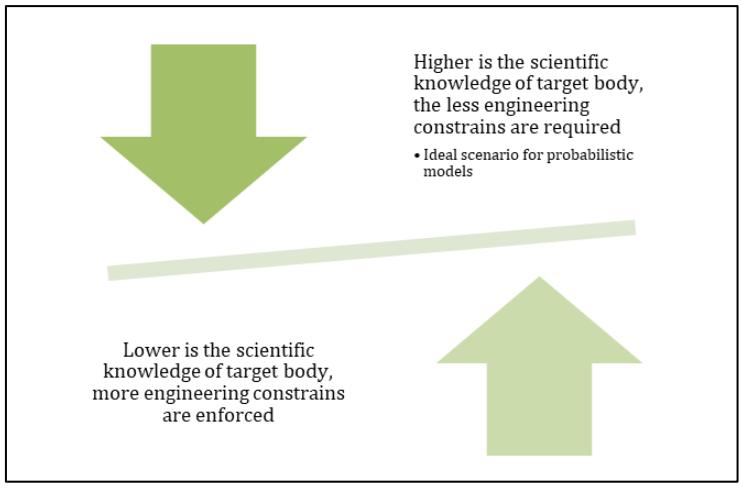


Figure 5-9: Planetary Protection risk - Science vs engineering

As space programs become more complex, a more advanced Planetary Protection strategy is needed. COSPAR and ESA already allow a risk-based approach for certain mission types, Cat. III orbiters to Mars, Icy Worlds and Earth Return, but implementing this fully requires development of new technologies and broad scientific international consensus. For example, recent advances in next-generation sequencing and metagenomics offer valuable new data that can help improve and update models that were originally developed decades ago.

Probabilistic requirements for contamination (i.e. overall target body threshold of 1×10^{-3} or 1×10^{-4} per mission) are in place to limit the probability that a biological inoculation event occurs to a habitable target body, within the period of biological exploration. A biological inoculation is the introduction of one or more viable organisms to a habitable body in the Solar system where such organism can replicate. Habitable bodies are those able to provide ingredients for life to exists (i.e. liquid water, chemical elements - CHNOPS, availability of energy)**.** Given current understanding of life on Earth, the limiting physical environmental parameters in terms of water activity and temperature that needs to be satisfied at the same time to allow the replication of terrestrial microorganisms are:

- Lower limit for water activity: 0,5.
- Lower limit for temperature: - 28° C.

These parameters have been derived by an international consensus process using latest scientific evidence and in the frame of Mars Special Regions. For more information on the reached outcome and the process followed, see [RD18].

The period of biological exploration (PBE) is the time (decades to centuries) during which a Solar system body is explored for signs of the origin of life and the history of prebiotic chemistry based on current scientific understanding. As such, protection of Solar system bodies is warranted during the PBE as applicable.

For Icy Worlds (i.e. Europa, Enceladus), **Category III and IV** missions, the probability of inadvertently causing a contamination event (i.e. biological inoculation) is set to 1×10^{-4} per mission for a period of biological exploration of 1000 years.

There is a link between the general COSPAR PP policy numerical guideline: " […] probability that a planetary body can be contaminated during the period of exploration should be no more than $1x10^{-3}$ " and the requirement for e.g. missions to Europa and Enceladus of inadvertent contamination of a subsurface ocean by viable terrestrial organisms set to $\leq 1 \times 10^{-4}$ per mission.

The former (1×10^{-3}) relates to the total probability of contaminating a given target body in the Solar system and assumes a total of 10 missions within a period of biological exploration. That translates into the latter requirement of 1×10^{-4} probability of contamination per mission that we see currently in Planetary Protection requirements for Icy Worlds.

5.5.3 Methods to assess compliance

As an example, this Section 5.5.3 and Annex A.2 include information of a probabilistic model that can be developed for Icy Worlds.

The probability of contamination for Icy Worlds includes a conservative estimation of (often poorly known parameters) such as:

a. Bioburden at launch.

b. Cruise survival for contaminating organisms.

c. Organism survival in the radiation environment.

d. Probability of landing on Europa or Enceladus.

e. The mechanisms and timescales of transport to a subsurface liquid water environment, and

f. Organism survival and proliferation before, during, and after subsurface transfer.

The objective of such probabilistic model is to determine if the probability of an individual spacecraft and lander contaminating another planet or moon with microbes from Earth within 1000 years is less than 1 in 10000, i.e. 0,00001. This value associated with our objective has been set by international consensous. The number itself is not, and cannot be, determined experimentally. As scientists and engineers our objective is achieved by demonstrating, to the best of our knowledge, whether a particular mission meets the criteria.

How to make assumptions

To calculate the probability for the spacecraft contaminating another world we need to use complex mathematical models, make numerical calculations, and rely on approximations. We also need to incorporate large amounts of uncertain knowledge. If we then try to place an upper and lower bound on the probability it is likely that the answers can always be 1,0 and 0,0, i.e. all values of probability are possible. However, it is also likely that the vast majority of the values that we estimate are clustered around a particular value. We can then make statements such as “the expected value of the probability is …” or “the probability that the value of the probability for the spacecraft contaminating another world is below the critical value is…”. Mission role is to provide the best estimate for the probability and the uncertainty associated with that estimate.

There is a very high risk that scientists, with the very best of intention, can distort the results of probabilistic calculations by “playing it safe”. When we are preparing estimates for inclusion into probability calculations in high-risk situations it is very tempting to adjust the data that we supply so that we are not taking any risk. For example, if we have nine routes by which a spacecraft can be contaminated then if all nine routes are judged to have a maximum probability for contamination to occur of 0,1 in 10000, then the total probability of contamination from all routes is 0,9 in 10000. On this basis our space craft is safe to launch. If just one person decides to raise their probability “just to be safe” to 0,12 in 10000 then the overall probability of contamination rises to 0.92 in 10000. So, the spacecraft is still good to go. If all nine people make the same decision of “just to be safe” and raise their probabilities to 0,12 in 10000, then the mission risk rises to 1,08 in 10000 and the mission cannot be launched. This can result in the mission being lost or an expensive re-engineering programme to bring the probability back below the critical limit. It is vitally important, therefore, that everyone involved does not change probabilities “just to be safe”. Leave that to those who are responsible for making the final decision.

When we analyse statistical data, we know that there is uncertainty. We can incorporate that into the analysis and provide clear advice to decision makers. It is not helpful if individual scientists distort their information "just to be safe".

To be noted that for many of the probabilities needed to evaluate the probability for the spacecraft contaminating another world, there is no correct answer. It can be impossible to run enough trials to obtain a statistically use estimate of a probability. Therefore, we need to depend on the opinion of experts. Any opinion is a valid belief about the probability and given two experts and you are likely to get two different estimates for a probability, and both are valid. Our aim needs to be to provide information that minimises the variation between experts about a particular probability.

The following tasks are foreseen as a method of compliance for probability of contamination requirements:

- **Task 1:** Assess microbes' survivability for missions to Icy Worlds environmental conditions, i.e. Europa, Ganymede, and Callisto.
- **Task 2**: Determine the existence of potential habitable zones and contamination transport on those worlds.
- **Task 3**: Develop and feed a probability model of contamination.

Metagenomics and molecular biology can be used for **Task 1** to address the functions of microorganisms and understand how they manage to survive certain stressors. Use of gene and genome-centric metagenomics is recommended (in addition to standard spore assay). A 2-track approach can be followed for metagenomics: reference based (genome catalogue) and de-novo approaches to target unknowns. In this respect, predicted phenotypes and survivability can be verified by laboratory experiments. Predicted (supervised machine learning) and verified (experimental) values can be used in a flexible probabilistic approach for Planetary Protection to determine the survivability of microbial contamination on a target body. Current and future methods, databases and Artificial Intelligence based classification tools can be frequently evaluated to allow continuous improvement to standard operating procedures (e.g. training and test data sets for phenotype predictions or the size and scope of the reference genome database).

Interpretations of surface features of icy moons is used to address **Task 2**, enabling hypotheses about their permeability and internal structure. Current knowledge and considerations allow up to date estimates of the ice thickness between the surfaces of icy moons and potential liquid water in the subsurface (Ganymede: 26 km; Enceladus: 21 km; Callisto: 200 km; Europa: 20 km). These estimates can be used in calculations to generate probabilities of contamination for subsurface water bodies following impact of a spacecraft.

Bayesian statistics is used to address **Task 3**. Assumptions can be made on the best possible scientific or engineering knowledge and cannot be too conservative or too adventurous in an attempt to accommodate risk. Microbes can be classified into groups that are defined by their properties and that the classification scheme can be redefined for each question needed to be answered. There are multiple routes to take when modelling the contamination of subsurface liquid water following impact on an icy world and the computational burden for each route can be different and decisions need to be taken based on resource availability.

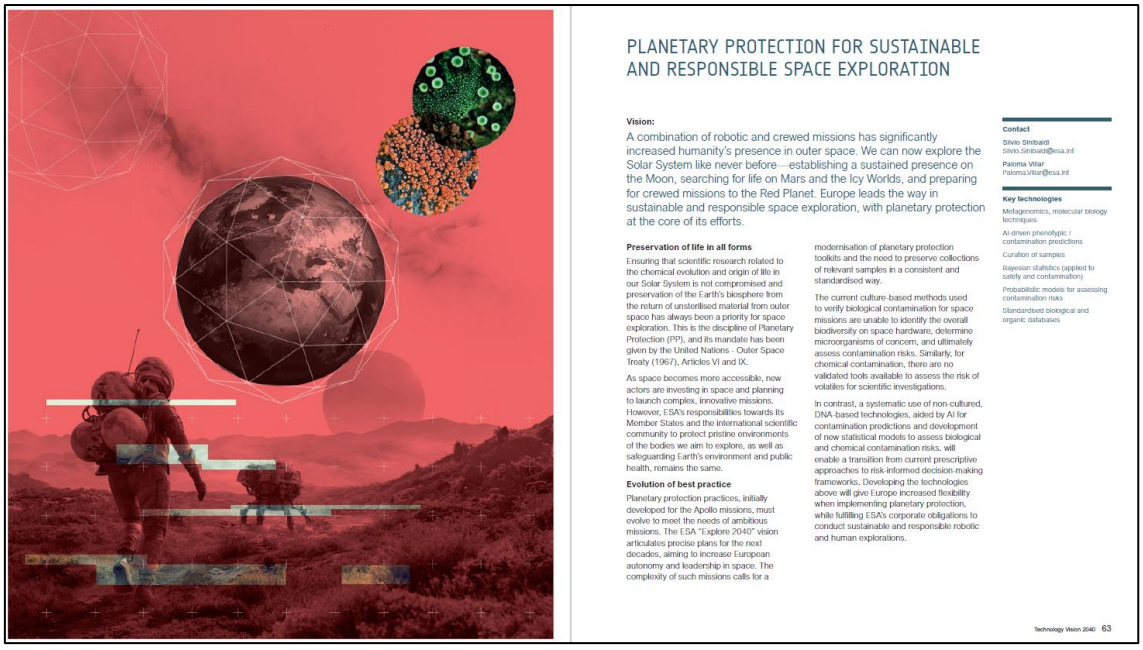
5.5.4 Mitigation measures

A practical example on how to tackle the probability of contamination is shown in Annex A.2.

Initial discussions within the COSPAR PPP have identified that the probabilistic approach is a sensible way forward for Planetary Protection, but additional work is needed to validate the methodology.

The best measure to allow development of probabilistic model for Planetary Protection is to invest on key technologies, knowledge advancement targeting major gaps and increase our understanding of still poorly known parameters, i.e. bioburden at launch, survivability of micro-organisms, transport mechanism of contaminants at target bodies.

This need has been highlighted at ESA in the Technology 2040 vision (ESA - Technology 2040), where Planetary Protection has been added as a "supporting technology" (see Figure 5-10).



PLANETARY PROTECTION FOR SUSTAINABLE AND RESPONSIBLE SPACE EXPLORATION

Vision:

A combination of robotic and crewed missions has significantly increased humanity's presence in outer space. We can now explore the Solar System like never before—establishing a sustained presence on the Moon, searching for life on Mars and the Icy Worlds, and preparing for crewed missions to the Red Planet. Europe leads the way in sustainable and responsible space exploration, with planetary protection at the core of its efforts.

Preservation of life in all forms

Ensuring that scientific research related to the chemical evolution and origin of life in our Solar System is not compromised and preservation of the Earth's biosphere from the return of unsterilised material from outer space has always been a priority for space exploration. This is the discipline of Planetary Protection (PP), and its mandate has been given by the United Nations - Outer Space Treaty (1967), Articles VI and IX.

As space becomes more accessible, new actors are investing in space and planning to launch complex, innovative missions. However, ESA's responsibilities towards its Member States and the international scientific community to protect pristine environments of the bodies we aim to explore, as well as safeguarding Earth's environment and public health, remains the same.

Evolution of best practice

Planetary protection practices, initially developed for the Apollo missions, must evolve to meet the needs of ambitious missions. The ESA "Explore 2040" vision articulates precise plans for the next decades, aiming to increase European autonomy and leadership in space. The complexity of such missions calls for a modernisation of planetary protection toolkits and the need to preserve collections of relevant samples in a consistent and standardised way.

The current culture-based methods used to verify biological contamination for space missions are unable to identify the overall biodiversity on space hardware, determine microorganisms of concern, and ultimately assess contamination risks. Similarly, for chemical contamination, there are no validated tools available to assess the risk of volatiles for scientific investigations.

In contrast, a systematic use of non-cultured, DNA-based technologies, aided by AI for contamination predictions and development of new statistical models to assess biological and chemical contamination risks, will enable a transition from current prescriptive approaches to risk-informed decision-making frameworks. Developing the technologies above will give Europe increased flexibility when implementing planetary protection, while fulfilling ESA's corporate obligations to conduct sustainable and responsible robotic and human explorations.

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Key technologies

Metagenomics, molecular biology techniques

AI-driven phenotypic / contamination predictions

Curation of samples

Bayesian statistics (applied to safety and contamination)

Probabilistic models for assessing contamination risks

Standardised biological and organic databases

Technology Vision 2040 63

Figure 5-10: Extract from Technology 2040 vision (Planetary Protection for sustainable and responsible space exploration)

The vision for Planetary Protection within "ESA technology 2040" advocates the transitioning from prescriptive approaches to risk-informed decision-making frameworks. In doing so, it identifies key technologies that need development, such as molecular biology, AI-driven phenotypic predictions and probabilistic models (e.g. Bayesian frameworks).

5.6 Mars special regions

5.6.1 Examples of Mars special regions requirements

Typical Mars special regions requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.3.

Clause 5.3.2.2.2 General requirements for Mars surface missions

c. *The supplier shall provide an analysis or assessment whether the spacecraft during nominal and off-nominal conditions has the potential to modify the local martian environment in a way that can create a Mars special region.*

NOTE Such an analysis or assessment is in particular important for spacecraft using radioisotope heat sources targeting areas with surface or sub-surface water ice.

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

NOTE This means at least no areas or volumes meeting the parameter definition for Mars Special Regions or already described features that can be treated as Mars Special Regions can be within the 3-sigma landing ellipse.

e. *The evaluations specified in 5.3.2.2.2c and 5.3.2.2.2d shall be based on the latest scientific evidence and include an assessment of the extent to which the temperature and water activity values specified for Mars Special Regions are separated in time.*

f. *The evaluation specified in 5.3.2.2.2d shall be updated during the mission whenever new evidence indicates that the landing ellipse and the operational environment contain, or are in contamination range of areas or volumes meeting the parameter definition for Mars Special Regions.*

5.6.2 Rationale for the requirement

Mars ''Special Regions'' are areas where stricter Planetary Protection measures are needed, as they have environmental conditions favourable to microbial growth. In particular, these refer to places warm and/or wet enough to support the replication of microbes carried by our spacecrafts.

The COSPAR Planetary Protection Policy defines Mars Special Regions as places interpreted to have a high potential for the existence of extant Martian life forms. The physical parameters that are needed to be satisfied at the same time to allow replication of terrestrial organisms have been established to:

- Lower limit for water activity (A_w): 0,5.
- Lower limit for temperature (T): - 28 °C with no upper limit defined.
- Timescale (t) within these limits identified in 500 years.

The current definition of special regions is the result of a multidisciplinary and multilayer activity led by the COSPAR PP panel and considering pivotal scientific inputs from MEPAG (Mars Exploration Program Analysis group), National Academies of Science, Engineering and Medicine and the European Science Foundation. Full details of the history and the international collaboration that led to the definition of special regions are contained in [RD20].

Spacecraft-induced Special Regions need also evaluation, consistent with the T and A_w limits and geomorphological features identified, on a case-by-case basis. Induced special region assessment is of particular importance in case of:

- Radioisotope heat sources are used, as they can heat up targeting areas with surface or subsurface water ice.
- Payload operations that can have the potential to access e.g. by drilling depths higher than 5 meters and create special regions due to temperature raise; or increase water activity during drilling operations. These cases need to be under the scope of the analysis made to ensure compliance with the requirements.

Features to be treated as Special Regions are:

1. Gullies (taxon 2 - 4) and bright streaks associated with gullies.
2. Subsurface cavities.
3. Subsurface below 5 metres.

4. Confirmed and partially confirmed Recurrent Slope Lineae (RSL).

NOTE For more information on classification of gullies and taxon, refer to [RD21]

5. From observational evidence for RSL, the following considerations can be used for initial assessment and classification:

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

   b) Partially confirmed: observed either incremental growth or recurrence

   c) Candidate: slope lineae that resemble RSL, but where observations needed for partial confirmation are currently lacking

The following features, if found, need to be treated as a Special Region until demonstrated otherwise:

1. Groundwater.
2. Source of methane.
3. Geothermal activity.
4. Modern outflow channel.

Observed features where a case-by-case evaluation before being classified as a Special Region is needed:

1. Dark streaks.
2. Pasted-on terrain.
3. Candidate RSL.

5.6.3 Methods to assess compliance

The method to verify compliance is by Analysis of the landing areas and Review of scientific literature and fixtures associated with special regions within the landing ellipse. The information reported in Section 5.6.3 is a summary of the method that has been used for establishing **Category IVb** missions (ExoMars Rosalind Franklin Module) and based on [RD22].

It is important that the evaluations specified in these requirements is based on the latest scientific evidence and include an assessment of the extent to which the temperature and water activity values specified for Mars Special Regions are separated in time. Nominal and off-nominal conditions need both evaluation in such an analysis.

It is important that focus is put on identification and description of geological, geomorphological features, made by visual inspection of high-resolution images to review potential of:

- Occurrence of water-related features on the base of global data and scientific literature.
- Geological-geomorphological setting of the area.

Analysis and inspection of high-resolution images are the most appropriate methods for investigating features potentially formed by water-related processes, such as unidirectional flows, ice solifluction, partial or intermittent melting, hydrothermal activity and water ponding. Visual inspection is particularly effective for identifying features that cannot be easily described by simple geometrical parameters or reliably detected by automated software.

Even features that are readily recognisable by visual inspection, such as impact craters or boulders, cannot always be mapped automatically without manual refinement. Particular attention should

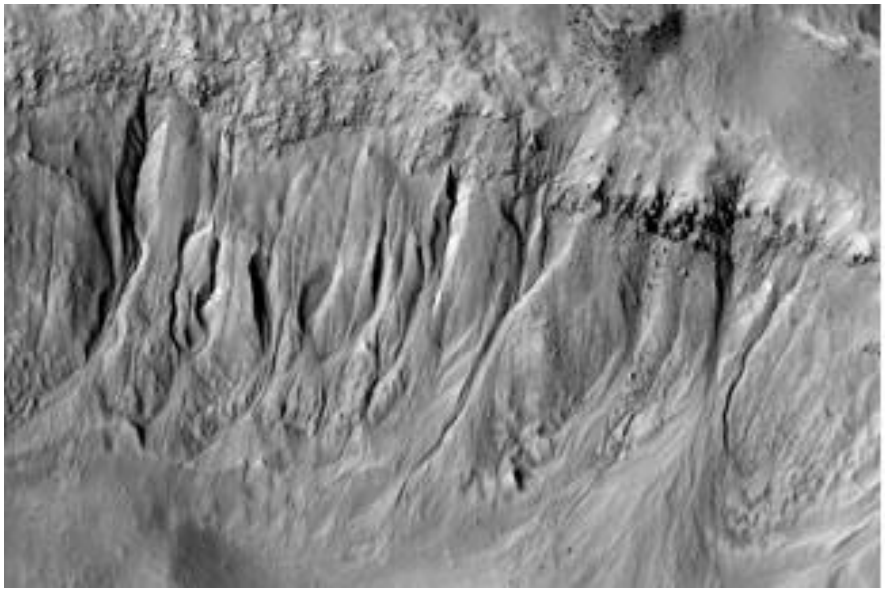
therefore be given to slopes and craters, with the search focused on features potentially formed by water-related processes.

In particular, the following features need searching in great detail:

Gullies, and bright streaks associated with gullies (Gullies are covered in salts, like chlorates, carbonates and sulphates. These minerals originate when water and rocks interact with each other resulting in the dissolution of rocks and, upon evaporation, a salt deposit appears.

- Figure 5-11).
- Recurring Slope Lineae (Figure 5-12).
- Pasted-on terrains.
- Others possibly water-related features (dark streaks, possible geothermal sites, fresh craters with hydrothermal activity, modern outflow channels, or sites of recent seismic activity).

In order to identify and describe geological features, for e.g. the HiRISE camera on-board NASA Mars Reconnaissance Orbiter, can be used and it is capable of acquiring planetary surface images of high resolution (about 25 cm/px). MOC-na images of NASA Mars Global Survey or spacecraft can also be considered, in order to verify possible variation at the surface over the time. MOC-na images resolution vary from about 1 to 7 m/px.



Gullies are covered in salts, like chlorates, carbonates and sulphates. These minerals originate when water and rocks interact with each other resulting in the dissolution of rocks and, upon evaporation, a salt deposit appears.

Figure 5-11: Example of Martian gullies, suggesting the presence of water at or near the surface

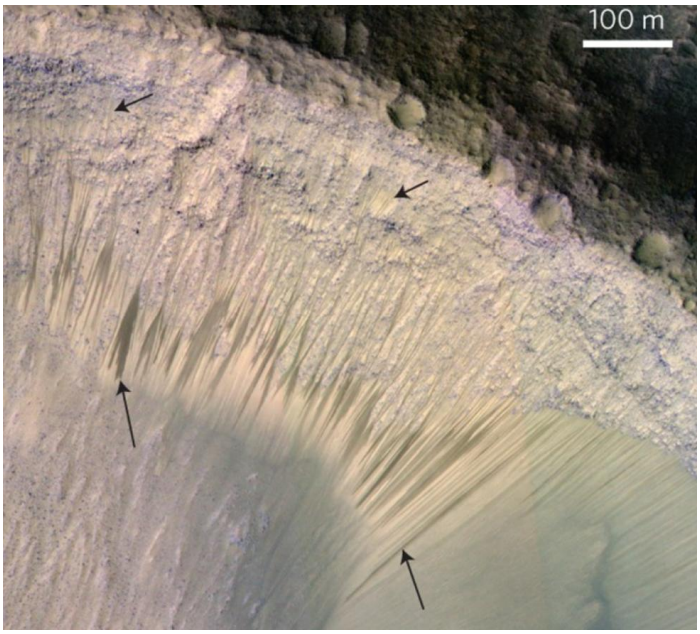


Figure 5-12: Recurrent Slope Linae (RSL) in Mars equatorial regions, taken from [RD23]. The arrows show locations of RSLs

As an example, IRSPS (international research school of planetary science) has performed a comprehensive study for ExoMars 2020 mission that was used as a method to assess compliance with special region requirements. More details can be accessed for ESA mission teams in [RD22] upon request.

The landing site selection for Rosalind Franklin mission is the Oxia Planum area of Mars, centred at about 335° East and 18° North (see Figure 5-13).

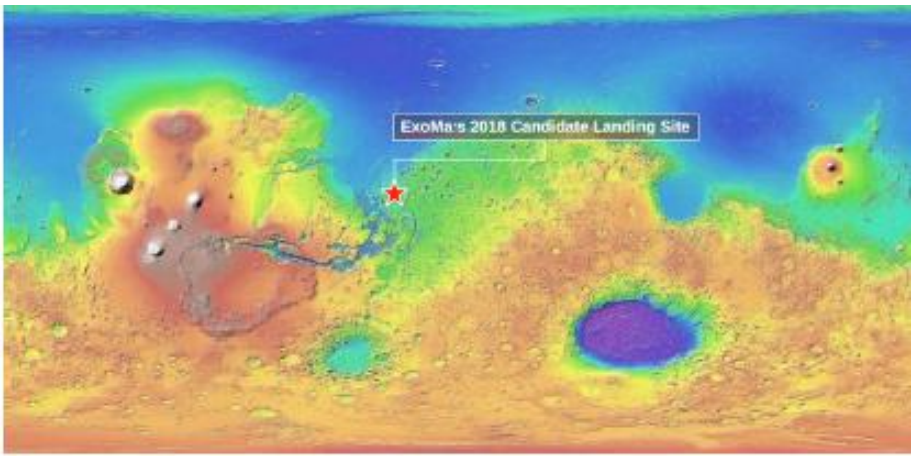


Figure 5-13: Location of the ExoMars 2020 candidate landing site on a global MOLA map of Mars

The landing ellipse, including the environmental terrain support analysis (ETSA) is shown in Figure 5-14.

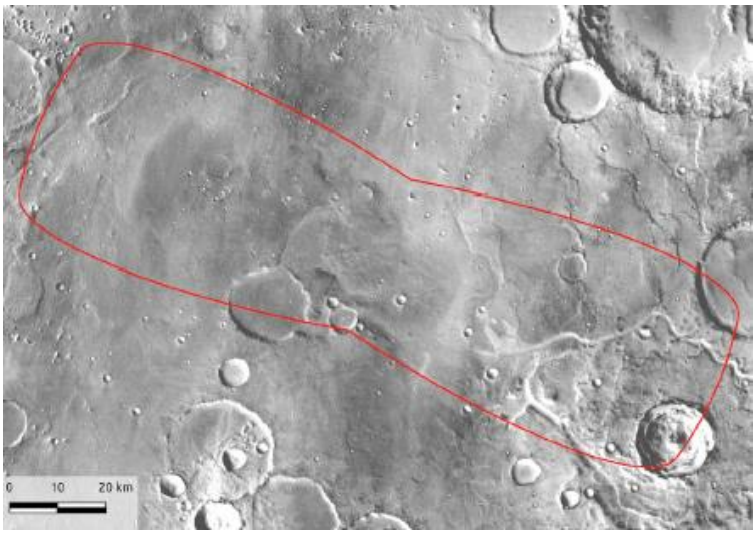


Figure 5-14: ExoMars 2020 mission landing site envelope for the ETSA activities. Base map is THEMIS IR Day mosaic

Geological, geomorphological fixtures potentially produced by water activity can be analysed, as per special regions requirements, with HiRISE and MOC-na images. In particular, images can be downloaded from the Mars Orbital Data Explorer (http://ode.rsl.wustl.edu/mars/index.aspx) of the NASA Planetary Data System Geoscience Node and analysed.

Figure 5-15 and Figure 5-16 show the map of images vs investigated area in Oxia Planum and the integration of the geological landing site map with the localization of prominent geomorphological features respectively.

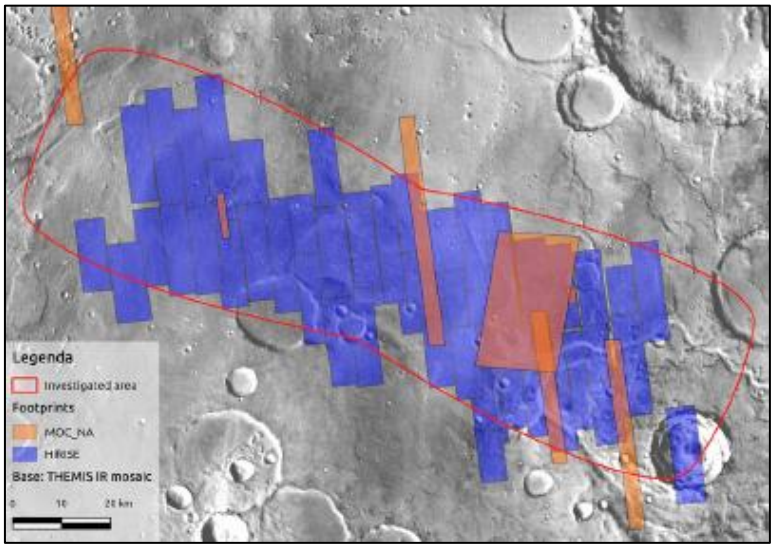


Figure 5-15: HiRISE images and MOC-na images map

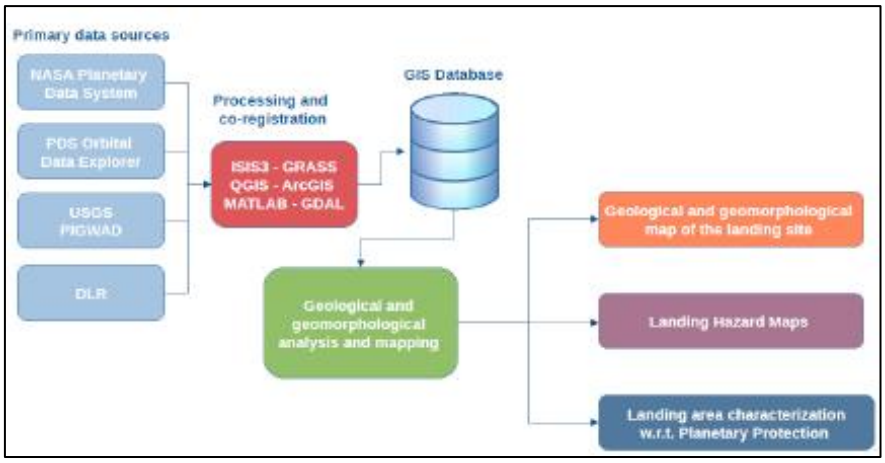


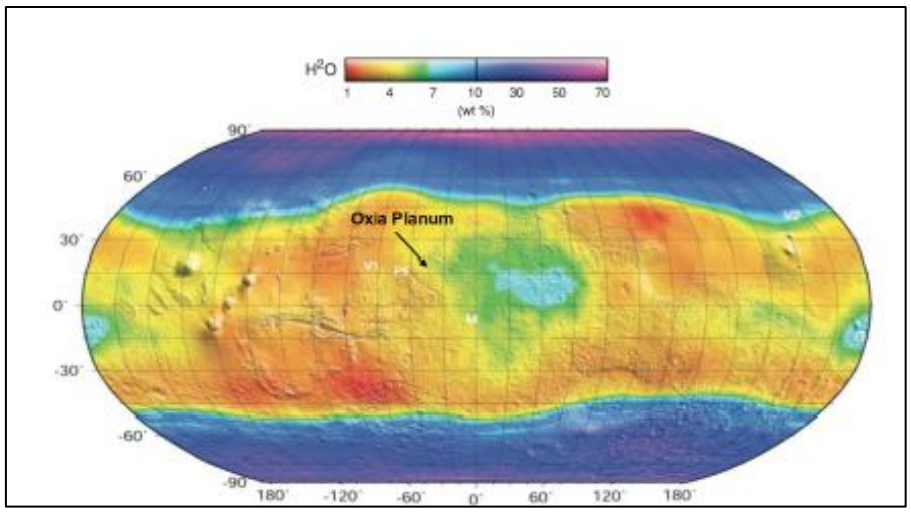
Figure 5-16: Analysis of the landing site with integration of dataset

Water related fixtures

Water content on near surface soil of Mars can be derived at global scale from the Gamma Ray Spectrometer instrument on-board NASA Mars 2001 Odyssey spacecraft. This instrument is able to investigate about the upper meter of Martian soil and its spatial resolution is very low (about 300 km/px), at least for the purpose of this analysis.

The near-surface soil of Oxia Planum appeared to be relatively dry, as shown in Modified from: PREVENTING THE FORWARD CONTAMINATION OF MARS. Committee on Preventing the Forward Contamination of Mars, National Research Council. (Credit: GRS instrument team, Lunar and Planetary Institute, University of Arizona).

Figure 5-17. The Gamma Ray Spectrometer data indicated that the amount of water in the near-surface soil (about the upper meter below the surface) was about 5 % in weigh. For comparison, Gamma Ray Spectrometer - GRS indicated that the amount of water occurring in the upper meter of soil of Gale Crater is about 7 %. Here, NASA Curiosity Rover measured a water abundance of about 1,5 % to 3,0 % in weight in in-situ analysis. Anyway, such analysis has been carried on the Rocknest surficial, loose, aeolian deposit.



Modified from: PREVENTING THE FORWARD CONTAMINATION OF MARS. Committee on Preventing the Forward Contamination of Mars, National Research Council. (Credit: GRS instrument team, Lunar and Planetary Institute, University of Arizona).

Figure 5-17 Global distribution and abundance of near-surface (top meter) hydrogen on Mars as inferred from the Mars Odyssey Gamma Ray Spectrometer data

Gullies and dark slopes streaks are considered water related features often developed quite recently in the geological history of Mars. Several work in past have been dedicated to map areas where such feature form. The following image (see Figure 5-18) indicates where such features have been found. Slope streaks occur in regions that have been known since the Viking orbiter missions to be thickly mantled with dust. Gullies occur at middle and high latitudes, where dust mantles are not so thick. The pink-shaded gully area is based upon the MOC team's survey of over 96000 MOC narrow angle camera images. The brown-shaded area, representing light and dark slope streak occurrences

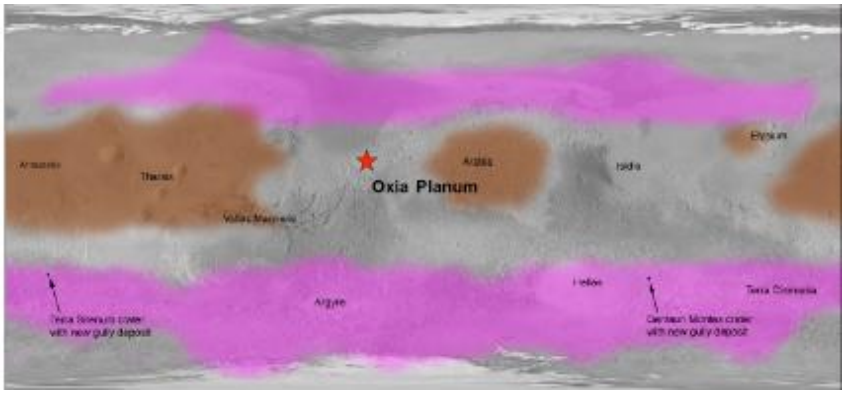


Figure 5-18: Map of Mars showing areas where gullies (pink), and light and dark slope (brown) occur. ExoMars 2020 landing site candidate is indicated by the red star

Geological and geomorphological fixtures

Oxia Planum is located on the south-western margin of Arabia Terra, between Mawrth Vallis and Ares Vallis. This area of Mars is late-Noachian in age and shows several outflows and channels converging toward Chryse Planitia. Several alluvial fans or deltas occur at the valleys outlets. The ExoMars 2020 putative landing site locates immediately northwest of a distributary system marking the termination of a slightly meandering channel belonging to the valley system of Coogoon Valles.

The investigated area was quite large (more than 6100 km^2), and characterized by a quite heterogeneous physiography, hosting several geological environments and morphologies (see Figure 5-19).

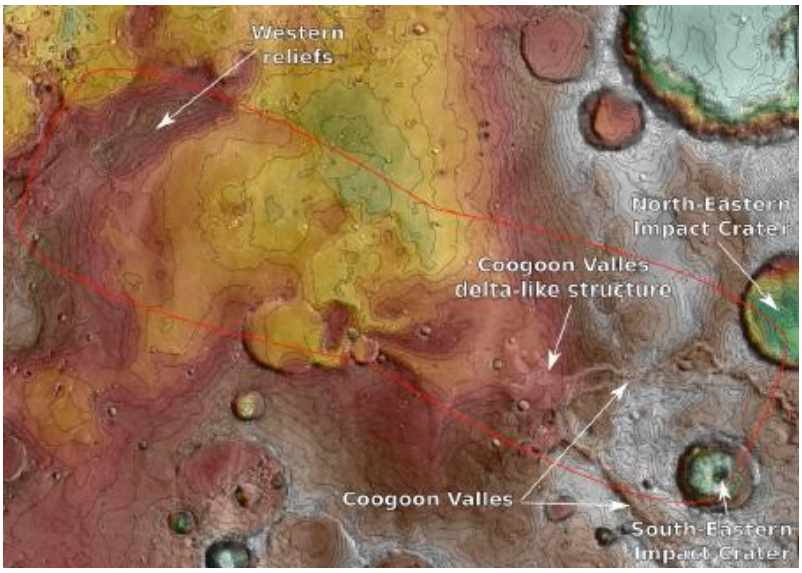


Figure 5-19: Geological environments and morphologies in the ExoMars 2020 landing site

The eastern-most portion is dominated by two large impact craters very different in age and morphology. The north-eastern impact crater (about 24 km in diameter) appears to be very ancient, characterized by a flat floor, rim almost eroded and ejecta mainly buried and eroded. The south-eastern impact crater (about 15 km in diameter),

A geological-geomorphological map was prepared and a different variety of geological environments, i.e. pseudo-fluvial and aeolian processes investigated. The inspection included Valley Walls and impact craters on the landing site. These did not show evidence of water activity in recent geological past, suggesting absence of special regions.

5.6.4 Mitigation measures

It is important that the process followed for identification of Special Regions on Mars aligns with project specific milestones, in particular in the frame of the so called "Landing Site Selection Working Group" (LSSWG), ad-hoc organised for a given mission. This is composed by a team of scientific and engineering experts from academia and industry, who recommend a landing site, balancing scientific needs for the mission, while adhering to engineering parameters.

Special regions need consideration from the LSSWG, as these inform the decision-making process when selecting the landing site. That is by highlighting potential Planetary Protection constrains in a timely manner, so that scientific return is maximised and programmatic delays are avoided.

It is important to understand that the evaluation for identifying potential Mars special regions needs periodic refining during the mission, whenever new evidence indicates that the landing ellipse and the operational environments contain or are in contamination range of areas or volumes meeting the parameter definition for Mars Special Regions. This process can be dynamic and not cast in stones, as new evidence on Mars surfaces can also lead to a re-evaluation of findings.

The knowledge (geological and geomorphological) acquired in previous missions (i.e. ExoMars 2016) can also be useful in the identification of special regions.

IMPORTANT: A special region evaluation needs to be performed for both nominal and off-nominal scenarios. It also needs updating during the mission whenever new evidence indicates that the landing ellipse and the operational environment contain or are in contamination range of areas or volumes meeting the parameter definition for Mars Special Regions.

5.7 Bioburden control

5.7.1 Examples of bioburden control requirements

Typical bioburden control requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.3.

Clause 5.3.2.1 Mars missions general requirements

c. Compliance to all bioburden requirements shall be verified pre-launch.

NOTE This verification is usually done on last physical access of the flight hardware or hardware elements, i.e., at delivery of flight hardware to next level contractor, delivery to launch site, and at the launch site prior to fairing closure.

e. One of the following conditions shall be met:

1. *The probability of impact on Mars by any part of a spacecraft assembled and maintained in ISO class 8 conditions, or better, is ≤ 1x10-2 for the first 20 years after launch, and ≤ 5x10-2 for the time period from 20 to 50 years after launch for nominal and off-nominal flight conditions.*
2. *The total bioburden of the spacecraft on Mars, including surface, mated, and encapsulated bioburden, is ≤ 5x105 bacterial spores.*

NOTE This requirement is also applicable for fly-by and gravity assist manoeuvres.

Clause 5.3.2.2.2 General requirements for Mars surface missions

a. The bioburden of the landed system on Mars shall be ≤ 3x105 bacterial spores on exposed internal and external surfaces.

NOTE Attention – for bioburden allocations to hardware and payload suppliers take into account re-contamination during different on-ground phases (e.g., testing at sub-system and system level, launch).

b. The average bioburden of the landed system on Mars shall be ≤ 300 bacterial spores/m2 on exposed internal and external surfaces.

NOTE 1 Attention – for bioburden allocations to hardware and payload suppliers take into account re-contamination during different on ground phases (e.g., testing at sub-system and system level, launch).

NOTE 2 Attention – for large spacecraft systems the average bioburden to meet requirement 5.3.2.2.2a needs to be much lower than 300 bacterial spore/m2

Clause 5.3.2.2.3 Surface missions with life detection

a. For surface mission with life detection one of the following conditions shall be met:

1. *The bioburden of the surface system on Mars is ≤ 30 bacterial spores on exposed internal and external surfaces, or at a contamination level driven by the nature and sensitivity of the particular life-detection investigations.*
2. *The average bioburden of the subsystems that are involved in the acquisition, delivery, and analysis of samples used for life-detection investigations is either:*

(a) ≤ 0,03 bacterial spores/m2, or

(b) at a contamination level driven by the nature and sensitivity of the particular life-detection investigations.

NOTE The contamination level driven by the particular life-detection investigation needs to be described in the Planetary Protection Plan DRR, Annex B.

b. The specific contamination level driven by the nature and sensitivity of the particular life-detection investigation described in 5.3.2.2.3a.1 and 5.3.2.2.3a.2(b) shall be subject to review and approval by the PPAA.

c. Recontamination prevention of the subsystems specified in 5.3.2.2.3a.2 and the samples to be analysed shall be in place until the end of the life-detection investigations.

Clause 5.3.2.2.4 Surface missions accessing Mars special regions

a. If a Mars special region is within the 3-sigma landing ellipse, the bioburden of the entire surface system on Mars shall be ≤ 30 bacterial spores on exposed internal and external surfaces.

b. If a Mars special region is accessed through horizontal or vertical mobility, one of the following conditions shall be met:

1. The bioburden of the entire surface system on Mars is ≤ 30 bacterial spores on exposed internal and external surfaces.

2. The subsystems which directly contact the Mars special region are sterilized to levels specified in 5.3.2.2.4b.1, and a method of preventing their recontamination prior to accessing the Mars special region is in place.

NOTE Example of accessing Mars special regions are by roving (horizontal mobility) or by drilling (vertical mobility).

c. If an off-nominal condition can cause a high probability of inadvertent biological contamination of a Mars special region by the spacecraft the bioburden shall be the following:

1. The bioburden of the entire surface system on Mars is ≤ 30 bacterial spores on exposed internal and external surfaces, and

2. The total surface, mated, and encapsulated bioburden level on Mars is ≤ 30 + 1,5 x 104 bacterial spores.

NOTE Example for off-nominal condition is a hard landing.

5.7.2 Rationale for the requirement

All bioburden constrains, identified in Section 5.7.2 are for Mars missions. The "bacterial spores" mentioned in the requirements are defined with respect to the number of microorganisms that can respire aerobically, survive a heat shock at 80 °C for 15 minutes, followed by rapid cooling, and can be cultured on TSA (Tryptic Soy-Agar) at 32 °C for 72 hours [35], [36].

Rationale: the international scientific community has chosen a bacteria representative of thermally resistant spores that are typically present on space hardware, as a "proxy" with which to measure the effectiveness of sterilization approaches, in particular dry heat microbial reduction. International scientists have chosen the spore of *Bacillus subtilis* variety *niger* (also called *Bacillus globigii* – BG) in studying sterilisation efficiency of flight hardware.

Spore formation is a survival strategy to escape temporarily unfavourable conditions. This is considered a dormant state that can last from tens to thousands of years and before returning to a vegetative growth state. Figure 5-20 shows the spore formation mechanism.

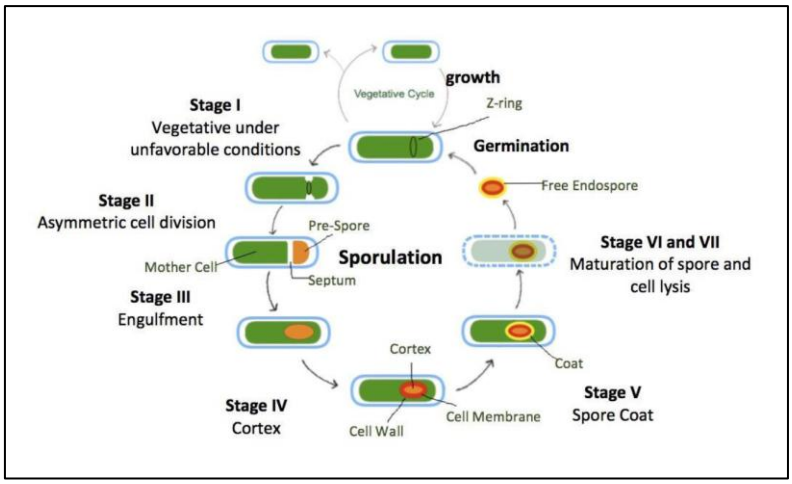


Figure 5-20: Spore formation stages, from [RD24]

A dormant, metabolically inactive stage, i.e. spores, is more resistant against challenging environmental conditions than a corresponding vegetative stage. External stressors like:

- Desiccation, encountered in cleanrooms.
- UV radiation, ionising radiation, high salt concentrations, oxidising compounds (encountered during journey in space to a target body or at actual target body).
- Aggressive chemical agents (acids, acetone, alcohols) used to clean space hardware.
- Heat, used to sterilise space hardware.

are all examples of environmental parameters that can induce microbes to form spores.

Figure 5-21 shows a typical morphology of a *bacillus pumilus* spore, with different protection layers, protecting the core and nucleoid.

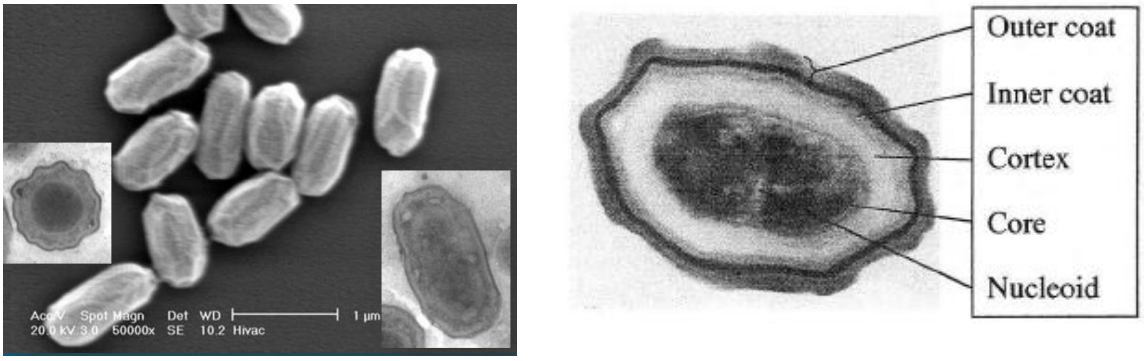


Figure 5-21: *Bacillus pumilus* under microscope, showing different layers of protection from environmental stressors

The bioburden thresholds currently defined for landing missions to Mars are derived from the number of spores (3×10^5) and densities (300 spores/m^2) calculated on the NASA Viking landers with >2000 biological assays and prior to sterilisation. These are still the values we see in our requirements today.

By meeting the current bioburden limit for Mars missions (as applicable), it is assumed that the biological control in place can be enough to reduce the probability of harmful contamination to 1×10^{-3}

during the period of biological exploration (PBE) for Mars (i.e. 50 years minimum). The 1 x 10^{-3} probabilistic target has been set by the international COSPAR PP policy as the numerical guideline to be used for protecting a planetary body.

To be noted that the total spore count for robotic missions to Mars accessing so called "special regions" (Section 5.6) are considerably lower than other areas on the Red planet. That is due to the potential of microbes to replicate on areas with Aw = 0,5 and T > - 28° C (as per special region definition) and based on the life as we know it.

For surface missions designed to detect potential extraterrestrial life, a more restrictive requirements in terms of spore count (30 spores) and spore densities (0,03 spores/m^2) can be levied place to avoid the so called "false positives", that is any unwanted organic contamination introduced to the sampling process, due to cross contamination in the sample under analysis, and affecting the validity of associated scientific results. These requirements are generally placed for so called Ultra Clean Zones (UCZ). Those are the areas involved in the acquisition, delivery and analysis of Martian samples for life detection. To be noted that the 0,03 spore/m^2 spore density requirement, has been derived by assuming an initial bioburden spore density of 300 spores/m^2 and a sterilisation (for e.g. by dry heat) to achieve log4 reduction.

Additional organic requirements are added for UCZs to protect Martian samples to be analysed. These requirements limit the maximum TOC (total organic carbon), which is the maximum terrestrial contamination level per organic substance class and per gram of Martian samples for life detection purposes. These values depend on the sensitivity of the instrument used for searching extraterrestrial life. For the ExoMars RFM mission for example, thresholds from monomers of Kapton, Mylar and PTFE, as well as fluorinated lubricants and any other organic compound have been set (TOC levels from tens to few hundreds of nanogram).

5.7.3 Methods to assess compliance

Compliance with the requirements can be achieved by testing space hardware, i.e. performing biological assessments as per Section 5.8 throughout the mission and at different AIT stages and before launch.

An alternative method to prove compliance with requirements is by analysis. This can be performed by demonstrating that a mission can have a probability of contaminating Mars of less than 1 x 10^{-4}. Contamination is intended as the introduction of one or more viable organisms that can replicate on Mars under favourable environmental conditions, in terms of nutrients, energy and presence of water.

It is important to note the origin of the 1 x 10^{-4} probabilistic requirements. In order to meet the overall objective of reducing the probability of contaminating Mars to 1x 10^{-3} during its period of biological exploration (as per COSPAR PP policy), one order of magnitude margin is taken for a single mission (assumed 10 mission during the PBE).

While there is no specific format for calculating the probability of contamination, this needs rigorous considerations of bioburden at launch (class and location on the spacecraft), survivability of micro-organisms during journey in space and under environmental conditions at target body, transport mechanism that can cause contamination event with replication of one or more viable organisms. Such analysis constitutes a performance based, risk-informed approach that needs review and approval from the ESA PPAA (Planetary Protection approval authority).

5.7.4 Mitigation measures

In this Section 5.7.4 focus is given to mitigation measures typically taken for Mars landers (**Category IV a-c**) that decide to meet the spore count and density requirements.

Planetary Protection measures are rigorously pursued from the very early phases of the mission. Limiting biological contamination requires PP considerations from the initial mission concept and space hardware design. Given the associated requirements described in this Section 5.7.4, it is important to consider the so-called triad of contamination, as indicated in Figure 5-22.

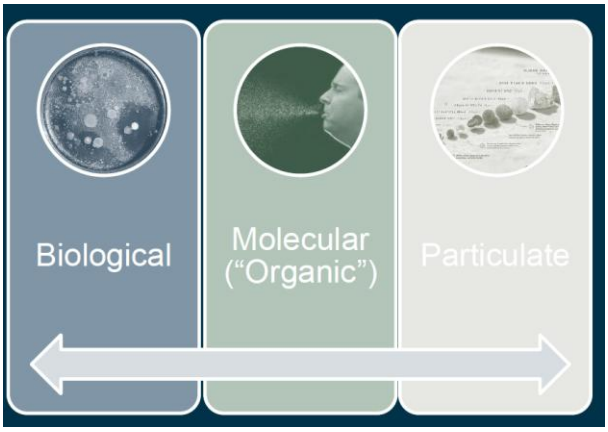


Figure 5-22: Triad of contamination considered for landers to Mars in Planetary Protection

Biological contamination control necessarily includes management of both molecular and particulate cleanliness, in addition to microbial. Examples of particulate contaminants are skin flakes, hairs, clothes (textile) fibres, etc.; and all of which can be passengers for microbes. Molecular contaminants usually include skin oil and grease, perspiration, saliva, fluid mucus (coming from sneezing, coughing), which is a carrier of microbial content. Abiotic organic sources such as adhesives, pottants, conformal coatings and certain polymers, used in high quantity in spacecrafts, can also be used as a growing media by microorganisms.

The Planetary Protection function in a project interfaces with multiple disciplines and can be involved at different mission stages. Figure 5-23 qualitatively shows the level of involvement and tasks throughout a given project lifecycle.

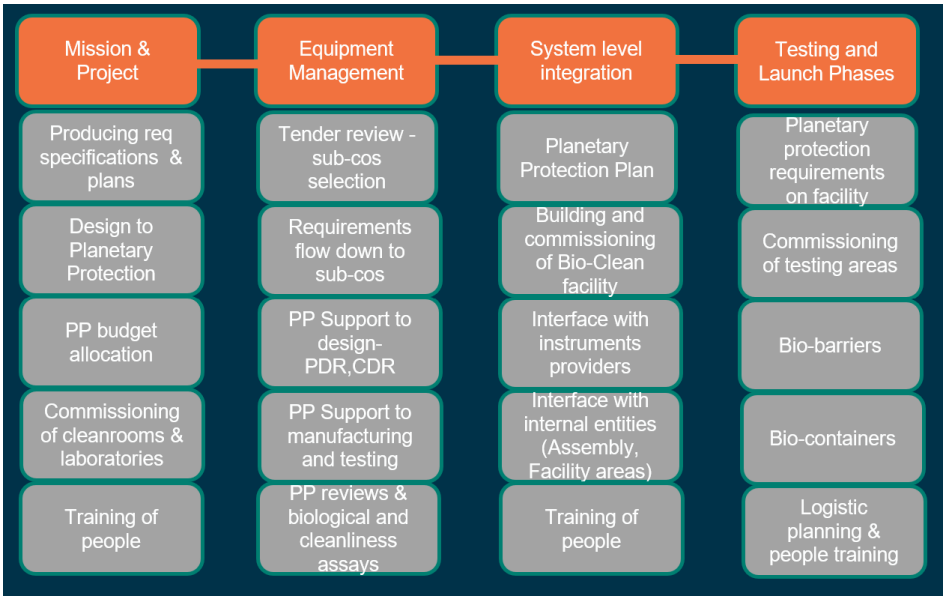


Figure 5-23: Notional chart showing Planetary Protection function involvement during different mission phases

Tackling the triad of contamination (microbial, particles and organics) is the preferred measure to mitigate the risk of exceeding associated bioburden requirements. In doing so, it is important the following aspects are considered in the PP implementation process:

1. Category assignment.
2. Generation of PP requirements, strategies and plans.
3. Recontamination prevention throughout the different mission phases.
4. Training of personnel.

Category assignment

The first step to initiate the Planetary Protection process is to assign the mission category: for landers, that is **Category IV**. However, it is important to note that, based on the mission description and objectives, 3 subcategories need to be considered and established:

- **Category IV-a**), for landers to Mars (not accessing special regions) and only mapping the surface, i.e. no search for life instruments.
- **Category IV-b**), for landers to Mars (not accessing special regions), but carrying search for life instruments, such as gas Chromatography and mass spectrometry.
- **Category IV-c**), for landers to Mars accessing special regions.

The categorisation process gives projects an indication of the amount of resources needed, how and where to allocate them. It can also drive specific requirements for the missions, see next sectionsd.

Generating PP requirements, strategies and plans

The creation of a requirement specification (tailored from ECSS) is the next step after the categorisation process. Requirements are generated by project and reviewed by the PPO/PPAA, as described in section 4.4.

Once requirements are generated, strategies need to be in place for the implementation phase. A key aspect when tackling PP constrains for a landing mission to Mars, is the creation of a bioburden budget. That is done by diligently allocating spacecraft total count and spore densities (i.e. 5×10^5 or 3×10^5, and 300 spores/m^2) to different elements or subsystems. It is important to take some margin and do not use the entire budget. A 20 % of the total number of spores is generally taken as buffer in case contamination events occur during the manufacturing and assembly of space hardware. That means, if project initial budget for a Mars rover element is 3×10^5 spores, only $2{,}4 \times 10^5$ spores are initially allocated to the different mission elements, keeping 6×10^4 spores (equivalent to 20 % margin) for potential contamination events that can occur for e.g. during testing or launch campaigns.

In creating the bioburden budget, bioburden accumulation throughout the different mission phases can be predicted: from the time of delivery of individual equipment, to system level integration, environmental testing and launch. In doing so, a good practice is to consider (as a minimum): planned sterilisation processes, cleaning activities with solvents, integration of sterilised items in bioburden-controlled environments. Data from previous missions (with similar or same category) can be an asset to accurately predict those values.

Table 5-6 shows a simplified example of how to allocate total amount of spores to subsystems throughout different mission phases.

It is extremely important to estimate the internal and external surface area of a subsystem. Reason being, after equipment assembly, it won't be possible to clean the internal surfaces during integration, testing and launch stages. That can result in different bioburden cumulation levels.

The total spores at delivery assumes an average bioburden density of 300 spores/m^2.

The total spores after integration assumes a re-contamination of 40 %. This value can change depending on cleanrooms used.

Thermal vacuum is considered a "dirty" test for bioburden recontamination. That is due to the re-pressurisation process, ideally performed through an HEPA filter with an inert gas (for e.g. nitrogen). During thermal vacuum test, subsystems "breath in and out". As a consequence, contaminants from the inside can be transferred to external surfaces. For that reason, it is a reasonable conservativism to assume a re-contamination during this phase of 100 %.

Vibration is typically performed in un-controlled environments. Bio-barriers are used to protect the hardware before, during and after testing. Other measures to mitigate contamination can be taken, as limiting the time at test as much as possible, limiting number of people, or commissioning (cleaning and assaying) the test area before introducing flight hardware. Nevertheless, a certain level of re-contamination is assumed. For this specific case only 30 % can be assumed, however, depending on the environment, it can change.

Bioburden reduction measures can be assumed after testing. The most used is called DHMR (Dry Heat Microbial Reduction). However, other means to reduce bioburden levels can be used, as described in Section 5.12. In the specific case of Table 5-6, a log 2 reduction has been assumed. That means, if my starting bioburden density is 1000 spores/m^2, a log 2 reduction reduces initial bioburden density to a factor of $10^2 = 100$. As such, bioburden density after log2 is equal to 10 spores/m^2.

Allocation to different subsystems can consider a margin, generally 20 %, in order to account for recontamination events or incidents that can occur during AIT operations. Encapsulated bioburden that is exposed to the Martian environment (e.g. wear products) is considered part of the accountable bioburden budget.

Table 5-6: Notional example of spore budget predictions and final allocation

Subsystem	Surface area			Total spores at delivery	Total spores after integration (assumed 40 % recontamination)	Total spores after thermal vacuum test (assumed 100 % recontamination)	Bioburden after vibration (assumed 30 % recontamination)	Bioburden reduction (log2)	Allocation to different subsystems (considering margin 20 %)
	Internal (m2)	*External (m2)*	*Tot (m2)*						
Structure	2	12	14	4200	5880	11760	15288	152,88	127,4
Thermal	1	8	9	2700	3780	7560	9828	98,28	81,9
Avionics	2	3	5	1500	2100	4200	5460	54.6	45,5
Harnesses	1	3	4	1200	1680	3360	4368	43,68	36,4
Entry Descent Module	12	18	30	9000	12600	25200	32760	327,6	273
TOTAL	18	44	62	18600	26040	52080	67704	677,04	564,2

A cleanliness and contamination budget (for particulate and molecular), similarly to a bioburden budget, is also created by the responsible CC&C (cleanliness and contamination control) engineer. Despite this is not a direct responsibility of the Planetary Protection engineer, it is extremely important that the CC&C and PP functions coordinate and exchange information about measures to be taken to meet final requirements. An obvious example on the importance of such coordination, is to avoid contamination events caused by volatiles (coming from outgassing products released by polymers, adhesives) leaching through UCZs and creating false positives.

Recontamination prevention throughout the different mission phases

Once flight hardware has been cleaned, assayed and sterilised to requirements as appliable and for a given mission phase, it is important to maintain that level over time and throughout the duration of AIT and launch. In doing so, a series of measures are taken including:

1. Frequent cleaning with isopropyl alcohol (IPA 70 %), bioburden monitoring of flight hardware, ground support equipment (GSE) in intimate contact with flight hardware and use of cleanrooms / bioburden-controlled environments until launch.
2. Use of “bio barrier” , i.e. covers or packaging materials to protect the hardware when it is not worked on, or in testing operations.
3. Use of bespoke bioburden, humidity and temperature-controlled transport containers, to move the hardware from one location/cleanroom to another.

Some examples of bio-barriers, used at different stages, are shown below.

Figure 5-24 shows the Rosalind Franklin Rover lifted during environmental testing operations.

NOTE 1 CP-STAT-100 packaging material was used as a bio barrier to cover the rover during its lifting.

NOTE 2 A sheet cover, made of aluminum alloy, is also wrapped with the same packaging material. The cover is used to prevent contamination (particles and grease/oil) that is potentially coming from the crane and as a result of fretting during lifting operations.

Figure 5-24: Rosalind Franklin Rover being moved from a portable cleanroom onto its trolley during environmental testing operations

Figure 5-25 shows a transport container being fit onto the ExoMars Rosalind Franklin rover (left) and the one for the Schiapparelli Entry Descent Module attempt in 2016 (right).

Figure 5-25:Transport containers examples (credit Thales Alenia Space Turin)

Figure 5-26 shows a "yurt" or tent used to protect the ExoMars Rosalind Franklin Rover during Electro Magnetic Compatibility (EMC) tests. The cover is made of the same fabric (cleanroom qualified) materials as the bunny suits worn by the operators in the picture.

An EMC test can last for days to weeks , as such that protection can last for quite some time, including during the actual EMC measurements. It is important to highlight that the material can interfere with

the actual EMC measurements taken during test. Before choosing the correct material option, compatibility tests are performed to make sure any signal deviation caused by the protection is accounted for.

The anechoic chamber used for EMC testing is typically considered a high risk for re-contamination aspects. That is mainly due to the wall cones, which are made of a porous foam, capable to absorb high amount of contaminants, that can subsequently be transferred to flight hardware due to cross contamination by touch.

Note in the picture in Figure 5-26 that some of the foams are completely wrapped up in bags (pink colour); these same bags can be tested prior to their use with FTIR – Fourier Transform Infrared analysis, to make sure the actual bag is not a source of organic contamination itself, and organic value are within acceptable limits.

Note also that the stall used to hold the EMC source (in the picture in Figure 5-26) is overwrapped with Kapton tape. Ans so the cables used to power the rover during test are overwrapped with CP-STAT-100 material (in the picture those cables lye on the floor, as they are running from outside the EMC room and connected to the actual rover).

Rationale for overwrapping these materials is to minimise the risk of organic contamination transferred by touch. The cables used for ground support testing activities are typically commercial, off the shelf items, with little scrutiny in terms of cleanliness and selection of materials.

It is typically a challenge to retrieve information about the materials used for external cable insulation. These are generally unknown polymers, plenty of plasticisers (i.e. phthalates) to make polymers softer. Overwrapping with known flight-qualified materials is good practice to avoid personnel holding those items by hand and subsequently transfer contamination by touching flight hardware with the same gloves. Avoiding unwanted organics is part of the overall PP strategy to tackle the triad of contamination (particulate, molecular, biological), as stated previously in this Section 5.7.4.

Figure 5-26: Electro Magnetic Compatibility test (credit Airbus UK)

Figure 5-27 shows typical set-ups during a thermal vacuum (TVAC) test. On the left side, it can be seen how the commissioned area is divided with metal panels, to avoid access from other un-controlled areas. A portable tent is also shown, which is typically used to prepare and equip flight hardware before

the actual TVAC test. On the right side of the picture, flight hardware (ExoMars Rosalind Franklin Rover) is ready to be tested on its support equipment. To be noted that, where possible, CP-STAT-100 bags can be used to protect as much as possible contamination coming from the loading platform. Ground support cables can also be overwrapped, similarly to mechanical or EMC testing.

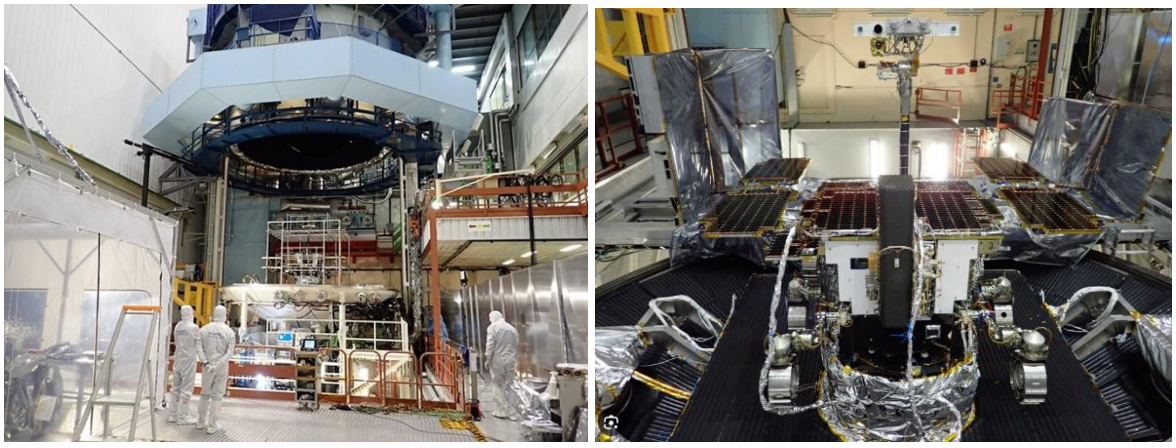


Figure 5-27: TVAC preparations during ExoMars rover environmental campaign at Airbus Toulouse, France

TVAC test can be considered one of the main causes of recontamination. Reasons include the presence of volatile products coming from numerous organic materials that outgas under hard vacuum, 10^{-5} mbar or better. Given the re-pressurisation process (from vacuum to ambient conditions), materials breath in and out, and cross contamination flow from internal to external equipment surfaces. Measures to prevent high re-contamination level during TVAC include:

1. **Use a 3 stage-process**:

    a) **1st stage (also called "chamber pre-bake").** After solvent cleaning of the facility and ground support equipment, generally performed with IPA 70 %, the TVAC chamber is set to its maximum temperature, for a minimum of 72 hrs. This phase, which can give a good biological reduction (if performed at temperature higher than 110 °C), can be monitored with TQCMs (Thermal Quartz Crystal Microbalance). These are thermally active devices, able to measure small quantities of mass (volatiles in this case) deposited on a quartz crystal using the properties of a crystal oscillator. Typically, the suggested minimum sensitivity level for a TQCM is 1,96 $ng.cm^{-2}$ Hz^{-1}. To achieve this sensitivity and long-term stability thermally matched 15 MHz crystals can be used. Before returning to ambient conditions, some stopping criteria can be requested based on specific project requirements. These can be established considering the deviation from linearity of the frequency measured by a TQCM. An example of this criterion is having real time measurements of TQCMs until the ration between the second and first derivative of the frequency is low enough, i.e. $f'' / f' < 1$ %, for a duration of 3 hours minimum. The final objective is to decrease the outgassing rate to almost steady state, i.e. to a point where additional time at temperature won't have significant effect in reducing contamination inside the chamber. It is recommended to fit the TQCM frequency using traditional mathematical models which describe outgassing kinetics (for example, Arrhenius law as described in ESA SP232). A minimum of 48 h data is recommended such as to consistently fit the frequency and establish the model. Once the data model is established, the ratio between the second and the first derivatives of the frequency (respectively in Hz.h-2 and in Hz.h-1) can be calculated versus the bake-out time (in h). For more information on how to analyse data from bake-out, "Analysis of bake-out monitoring data TEC-QT/2014/344" can be consulted.

b) Along with TQCM measurements, it is important to also consider the use of molecular witnesses (for e.g. zinc-selenide material) and particle fall out (PFO) plates. Typical measurements for a "clean" chamber on a molecular surface witness plate after pre-bake (measured according to ECSS-Q-ST-70-05) are in the order of few tens of ng/cm^2, for e.g. up to 50 ng/cm^2, when values are brought back to 24 h exposure of the witness plate. Value considers the aggregate of the four contamination rates determined regarding the four standard contaminants specified in ECSS-Q-ST-70-05 (esters, hydrocarbons and two types of silicones). For particulate, the obscuration factor measured by photometry in accordance with ECSS-Q-ST-70-50 on the PFO collection plate is typically < 275 ppm for a "clean chamber", when brought back to 24 h exposure of the collection plate.

c) **2nd stage (also called "blank" bake)**. The same TVAC conditions (both high and low cycles as applicable), that can be used during the flight hardware TVAC are used in the chamber "blank", BUT without adding any flight hardware. Blank duration is typically 48 hours and, despite TQCMs can also be added, use of molecular - MOC and particulate fall-out or PFO witness plates is the preferred method to monitor contamination inside the chambers. The maximum contamination levels for MOC and PFO are defined in the PP & CC plans. During the blank bake, it is recommended to add any GSE (mechanical or electrical) that can subsequently be used during the flight hardware TVAC test. This can include the holder where flight hardware is positioned during TVAC, or the thermocouples installed onto flight hardware at the next step.

d) **3rd stage (actual TVAC of flight hardware)**. Only after the chamber has been cleaned and certified as per previous stages 1 & 2, flight hardware can be introduced. It is important that among all those steps, the chamber is maintained with its door closed as much as practical, personnel working in the area is reduced to minimum, clear indications that a cleanliness sensitive process is running are in place.

e) In this phase, flight hardware is located inside the chamber, the process runs with use of one or more TQCMs in the field of view of flight hardware. MOC and PFO plates are used to monitor the chamber. It is extremely important that, after the process is completed flight hardware is immediately secured in bioburden-controlled environment and spends as little time as possible without use of bio-barriers.

2. Scrutiny of all materials of the chamber. Some of the harnesses used by the chamber provider can be commercial, off-the-shelf items that can be detrimental for flight hardware cleanliness. It is not uncommon to see connectors that are cadmium plated (forbidden material, it also sublimes under vacuum), insulation of cables that are not space qualified (i.e. PVC or with high quantity of plasticisers), or use of silicone greases on the chamber door to improve vacuum levels. The TRR – Test Readiness Review is generally the gate review where questions are asked about chamber materials and overall TVAC strategy.

3. Avoid the use of oil pumps due to back streaming of oil vapours; and ion pumps, as they generate a magnetic field. Preferred pumps are turbomolecular or cryogenic (Helium).

4. Use of a Cold Trap (designed to be easy to clean and avoid shedding /retention of particles. This is actively cooled down to reduce the risk of contamination and improve the vacuum cleanliness. It is important to maintain the temperature of the Cold Trap colder than any other surface within the chamber. That way contamination can condense on this surface. It is good practice to locate the Cold Trap in a straight view of flight hardware.

5. Keep the re-pressurisation rate low enough to ensure there is minimum risk of turbulence within the chamber with subsequent spreading of contaminants onto the hardware. Typical re-pressurisation rate can be < 20 mbar/min.

6. Use of HEPA 0,2 micron filters during re-pressurisation.
7. Avoid use of solvent cleaning after TVAC, as chemicals can be trapped in materials for e.g. paints.
8. Clean and biological assay chamber shroud and internal-facing side of doors can be assayed before the chamber pre-bake.
9. Where necessary, locate proxy samples, that have been pre-sterilised by autoclave next to the flight hardware. These can be assayed after TVAC, with any bioburden found to be assumed coming from re-pressurisation. The assay results can be used to confirm that there are still 0 colony forming unit (CFUs) on the proxies, which means no re-contamination happened from re-pressurisation).

Use of portable cleanrooms is part of recontamination prevention strategies. These can be utilised during testing and launch operations, for e.g. during preparation activities before environmental testing and where the cleanliness of the overall area can be problematic for flight hardware. Examples are shown in Figure 5-28.

Figure 5-28: Portable tent examples used in cleanrooms during ALD (Analytical Laboratory Drawer) and ExoMars 2016 EDM (Entry Descent Module) activities

For Ultra-Clean Zones (s), where Mars sample contamination is a driver, Planetary Protection levels can be quite restrictive (0,03 bacterial spores / m^2 and total organic carbon in the orders of tens to hundreds of nanograms). In these cases, additional precautions can be taken, including an initial log 4 bioburden reduction, use of ISO 5 cleanrooms to prevent recontamination and subsequent handling inside sterile gloves box trains (GBT) as the one shown in Figure 5-29. Ultra-cleaning with accelerated CO2 snow and over-pressurisation with dedicated ground support equipment needed during assembly and testing of UCZs.

Figure 5-29: Globe box train in TASI Turin

Training of personnel

It is important that a training program is defined for all personnel directly or indirectly involved in Planetary Protection projects. It is good practice to establish different levels of courses, targeting relevant functions and expertise within the project.

A first level of training or **Level 1** can be provided to all mission teams. This can be focused on:

a. Giving general information about the legal framework and importance of adhering to Planetary Protection requirements (COSPAR PP panel, Outer Space Treaty).

b. Providing general awareness of mission categories, understanding of main Planetary Protection constrains.

c. Discussing ramifications to specific disciplines (system engineering, materials and processes, cleanliness and contamination, procurement, mission analysis).

d. Highlighting the importance of Planetary Protection involvement from early phases to allocate effectively project resources.

A deeper **Level 2** PP training can be provided for all personnel working in bioburden-controlled cleanrooms. This course includes:

a. Same information as indicated in **Level 1**.

b. Giving background knowledge on bioburden contamination and control, sterilisation procedures, how to handle sterile items, particulate and organic contamination.

c. Providing instructions on how to behave and work in bioburden-controlled environments, including practical examples on what to do in case of contamination events, or the speed when walking etc.

Planetary Protection **Level 3** courses can be provided for the supervisors designed to control the good implementation of the activities related to PP in bioburden control environments. The content of such a training typically includes all the information for level 1 and 2, in a more detailed and deeper manner.

Ad hoc training can also be organised for cleaning staff responsible to clean bioburden-controlled areas. It is extremely important that cleaning personnel is trained for:

a. Dressing in correct garments.

b. Frequency of cleaning.

c. Using ONLY approved chemicals for a given facility. To avoid microbial adaptation/resistance to one single chemical, it is good practice rotate 2 or more cleaning solutions. As such, it is important to establish an agreed weekly cleaning program.

d. NOT cleaning flight hardware, as chemicals used for bioburden-controlled environments differ from those used for flight hardware. For the latter, cleanroom chemicals can damage it, as most likely too aggressive for flight surfaces.

e. NOT cleaning ground support equipment (GSE). Exceptions can be made for racks or support equipment that won't be in touch with flight hardware and providing previous acceptance from project / PP function.

f. Cleaning in the correct sequence, which is typically from cleaner to dirtier areas, and drier to wetter.

Similar ad-hoc training can also be made for external contractors who can access bioburden-controlled environments occasionally, i.e. inspecting lifting devices, changing light bulbs, servicing instrumentations, etc.

It is good practice to also provide training to Security Personnel, who are not necessarily aware of Planetary Protection constrains and can access bioburden-controlled environments overnight or in late shift, without proper cleanliness supervision.

A training record can be established and kept up-to-date, and access granted only to personnel who have received the appropriate training.

Training only is not enough for guaranteeing good cleanliness practices. Refreshing sessions and/or daily briefing tailored for specific activities inside cleanrooms are important. These aim to increase awareness, communication and dialogue among project teams.

A "buddy system" with colleagues/personnel checking each other's, can be a good way to check for e.g. whether the dressing procedure is respected (see Figure 5-30).

Figure 5-30: Examples of pretty bad (left) and good (right) bioburden control attire

Explaining the "Why", the "DOs" and" DON'Ts", acceptable behaviours and attitudes inside bioburden-controlled environments, limit the number of personnel working at the same time on flight hardware are all good practices that can be explained in trainings.

Figure 5-31 and Figure 5-32 show some cleanroom actions.

Figure 5-31: Best practices followed by personnel inside bioburden control cleanrooms (credit: Airbus UK and Thales Alenia Space)

The operator on the left is touching the bunny suit with his gloves. This can cause cross contamination of hardwware if gloves were not changed. The operator on the right side is touching with his knees the floor. Note that certain activties cannot be performed without a certain level of cross contamination. However, a clear counter-mesure for avoiding bunny suit contact with the floor, can be placing a layer of CP-STAT-100 packaging material on the floor or change the bunny suit after the completion of the assay activity.

Figure 5-32: Examples of personnel operation in cleanrooms

Break up & Burn up analysis (BuBu)

The breakup and burnup analysis (BuBu) is a measure used in Planetary Protection for bioburden reduction purposes. It aims to achieve sterilisation (or a given bioburden reduction) using the heat generated by thermal dissipation on hardware subject to breaking and burning onto the Martian atmosphere.

The BuBu analysis can be used to demonstrate sterility of certain elements, i.e. Carrier Modules, if able to reach temperature of > 500°C for 0,5 seconds, after separation from Entry and Descend Landing Modules (EDLM). Such temperature is able to ensure complete degradation of organic molecules by breaking C-C bonds. A certain bioburden reduction can also be claimed if temperature reaches 200°C for 20 seconds.

The BuBu analysis typically consists of two break-up stages: the first one is when initial elements or equipment, i.e. Solar arrays, are initially detached and separated; the second is when a catastrophic failure of the remaining spacecraft happens. A Mars atmosphere model providing density as a function of altitude is an essential input for the analysis.

The final report typically includes information on:

1. Items that pass sterilisation criterion (500°C or higher for 0,5 seconds minimum) for the most pessimistic assumptions during entry phase (velocity and angle).
2. Those reaching temperatures higher than 200°C for more than 20 seconds, as well as
3. The full debris catalogue containing those parts that cannot reach sufficient temperatures to claim any type of bioburden reduction. In the latter case, assumption based on bioburden assays performed during AIT or use of witnesses/representative mock ups can be used to estimate their bioburden levels.

5.8 Bioburden assessment

5.8.1 Examples of bioburden assessment requirements

Typical bioburden assessment requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.4.

Clause 5.4.2 Bioburden assessment

a. *Bioburden assessment on flight hardware shall be performed, using procedures "Swab assay 1 (standard swab assay)" or "Wipe assay 1 (standard wipe assay) "as per procedures D.1 and E.1 from Annex D and Annex E of ECSS-Q-ST-70-55*

b. *If direct assays are not possible, estimation of the flight hardware bioburden using the highest number in the respective category of Table 5-1 shall be used.*

NOTE The encapsulated bioburden values in Table 5-1 cannot be used for ALM produced hardware.

Table 5-1: Bioburden estimation

Bioburden type	Specific environment	Bioburden value
Average encapsulated spores density (i.e. if no differentiation between electronic and non-electronic piece parts is made)	Non-metallic parts of the spacecraft:	130 spores/cm³
Source specific encapsulated spore density	Electronic piece parts:	3-150 spores/cm³
	Other non-metallic materials:	1-30 spores/cm³
Source specific enclosed surface spore density, e.g. a box closed in the specific environment	ISO class 8 cleanroom, highly controlled:	500-5000 spores/m²
	ISO class 8 cleanroom, normally controlled:	5000-10^5 spores/m²
	Uncontrolled environment:	10^5-10^6 spores/m²
Average surface spore density for cleanroom classes "in operation" (exposed and mated but non-encapsulated)	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 environment:	10^5 spores/m²
NOTE 1: Manufacturing processes can potentially be used to claim a lower encapsulated bioburden either through high temperatures (see ECSS-Q-ST-70-57 for relevant specifications) or control of manufacturing environment. NOTE 2: Normally controlled: use of gowning equivalent to the specific cleanroom class. NOTE 3: Highly controlled: bioburden control of cleanroom by use of full body coverall, hood, face mask, gloves and boots, restricted access and dedicated cleaning and microbial sampling.		

c. *Use of assay procedures not described in Annex D, Annex E, Annex F and Annex G of ECSS-Q-ST-70-55 shall be approved by the PPAA.*

d. *The number of samples to evaluate the bioburden for the flight hardware shall be agreed with the PPAA and meet the following conditions:*

1. *Five swabs for each surface area on flight hardware of 0,1 m2;*
2. *A proportionate number, but at least one swab, for each surface area on flight hardware smaller than 0,1 m2;*
3. *One wipe for each surface area on flight hardware in the size range of 1 m2;*
4. *Two wipes for each individual surface area on flight hardware per 10 m2.*

NOTE *Although the sampling plan is specific to the size and geometry of the sampled hardware, sampling at least 20 % of the surface area is a reasonable guideline. To reduce the conservatism in the overall bioburden calculations it is better to sample larger surface areas.*

e. The methods for calculation surface bioburden and bioburden densities shall be agreed with PPAA.

f. Bioburden prior to the application of a bioburden reduction procedure shall be established by using procedures specified in requirements 5.4.2a or 5.4.2b.

5.8.2 Rationale for the requirement

The rationale for having bioburden assessment requirements is to ensure that methods and procedures are in place to control microbial contamination on spacecrafts and cleanrooms. The detection of micro-organisms for Planetary Protection purposes and missions to Mars is based on cultivation of "proxies", *Bacillus subtilis* (*niger*) - like bacteria.

Quantitative examination of flight hardware surfaces can be done with swabs or wipes with the methods describe below.

5.8.3 Methods to assess compliance

The ESA spore assay procedure for flight hardware and cleanrooms is ECSS-Q-ST-70-55.

The standard swab and wipe assays target mesophilic aerobic spores and bacteria that can survive heat shock, with 80°C x 15 minutes and rapid cooling to 0°C. Below a description of those methods

Use of swabs

The general process flow chart is shown in Figure 5-33:

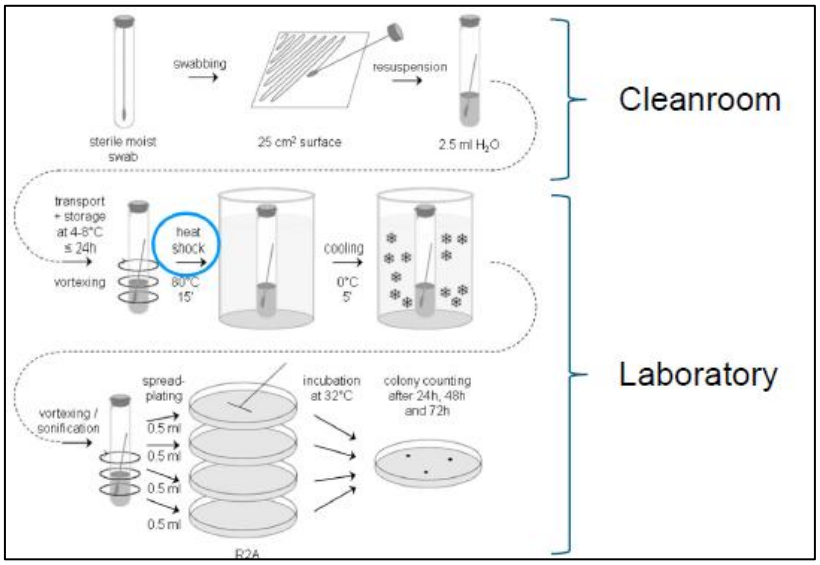


Figure 5-33: Flow chart for standard swab assay (operations performed in cleanroom and laboratory are identified in the Figure 5-33)

For sample collection, swabs can be used to represent surface areas lower or equal to 25 cm², for example 5 cm x 5 cm.

A sterile swab, type Nylon COPAN FLOQSwab 552C (Figure 5-34) is removed from its container and its head moisten in a test tube with sterile water, ASTM type IIB. Excess moisture from the swab is removed against the interior wall of the tube.

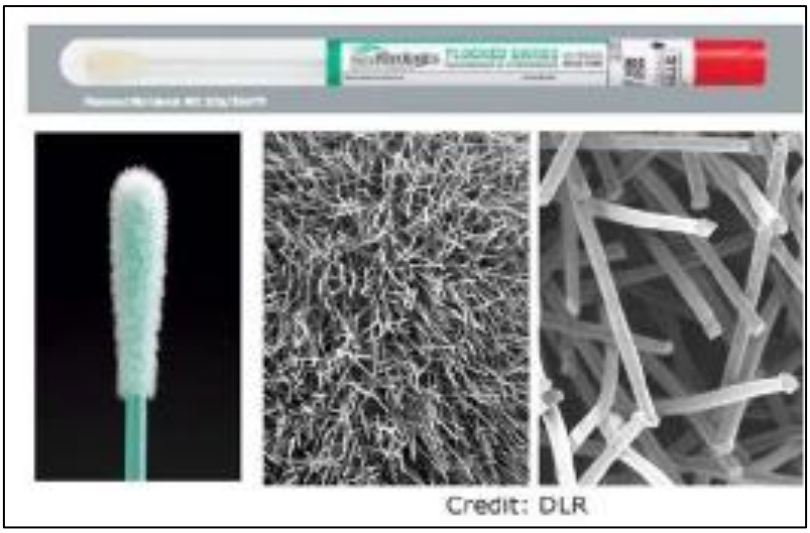


Figure 5-34: Nylon COPAN FLOQSwab 552C used of ESA spore assay (swab)

The swab is held so that the handle makes about a 30° angle with the surface to be sampled. While moving the swab in one direction, the head of the swab is rotated slowly and thoroughly over a measured 25 cm2 surface area. After that, the linear direction of the swabbing motion is changed to 90° and again the surface is thoroughly swabbed. A third coverage of the surface is completed by again changing the direction of the swabbing motion by 135°. Visual of the swab technique used is shown in Figure 5-35.

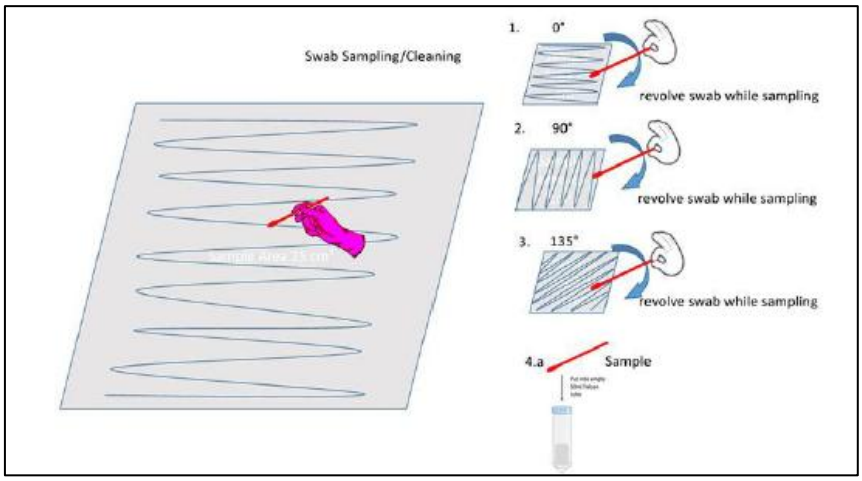


Figure 5-35: Sketch showing the technique for sampling surfaces with swabs

After that, the swab head is returned to a tube containing 2,5 ml sterile water, by breaking the swab shaft at the breakpoint.

Samples are transported to the laboratory and stored at 4 °C – 8 °C, and process performed within 24 hours.

The area to be assayed is not always even. On the contrary, difficult to reach areas are very common when sampling flight hardware. Some examples are shown in Figure 5-36.

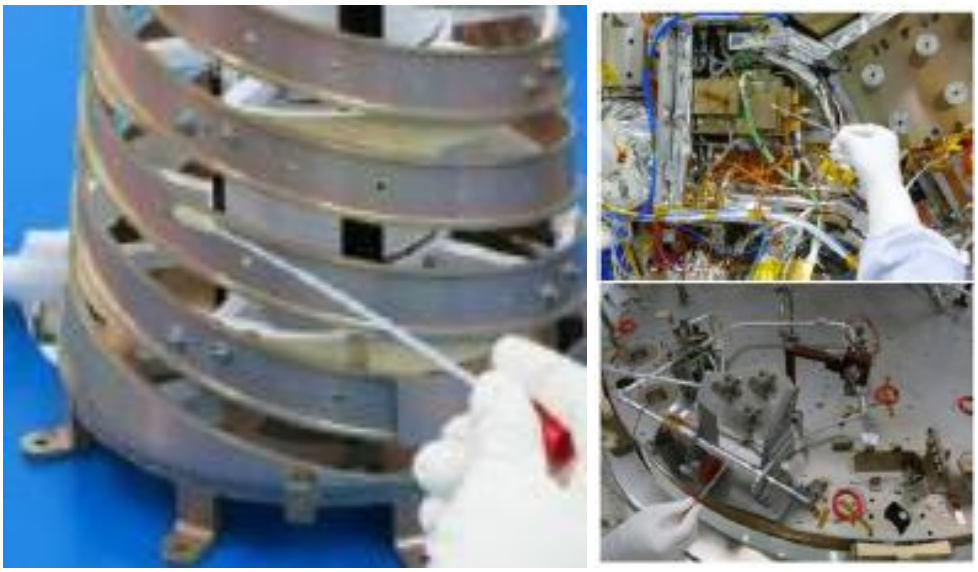


Figure 5-36: Examples of personnel sampling flight hardware in cleanrooms with swabs

Two people are needed to perform the sampling. The assistant typically holds the vials, identify the samples, record meticulously the location of the assays, take pictures of the areas being sampled as it happens. The assay taker can rinse the swab in sterile water and perform the assay in a given location. Sterile gloves are changed frequently as applicable. Figure 5-37 and Figure 5-38 show an example of worksheet and personnel at work performing the swab assay respectively.

A "field negative" is typically taken each ten or fewer samples collected by moistening the head of a sterile swab in sterile water and put it back the swab on its container (without assaying any surfaces).

Hardware	Sample	Location	Sampled by *
Landing Platform	S1	gold part of the ramp left -y	A
	S2	gold part of the ramp right -y	T
	S3	gold part up left -y	A
	S4	gold part down right -y	T
	S5	gold part of the ramp right +y	A
	S6	gold part of the ramp left +y	T
	S7	gold part up right +y	A
	S8	gold part up left +y	T
	S9	FC	T
	S10	solar array +y-z #1	A
	S11	solar array -y-z #1	T
	S12	solar array +y-z #2	A
	S13	solar array -y-z #2	T
	S14	solar array +y+z #1	T
	S15	solar array -y+z #1	A
	S16	solar array -y-z #1 (repeat)	A
	S17	solar array +y+z #2	T
	S18	solar array -y+z #2	A
	S19	FC	A
Solar array of Rover Module (SA/RM)	S20	SA peninsula 1st row 1 cell	A
	S21	SA peninsula 3rd row 4 cell	V
	S22	SA main panel right row (external) 1st cell	V
	S23	SA main panel rear right row 1 last cell	A
	S24	FC1	A
	S25	FC2	V
	S26	SA rgight 1sr row 5th cell	V
	S27	SA right 1t row 17th cell	A
	S28	SA right 2nd row first cell	v
	S29	SA right rear row first cell	A
	S30	SA right 3rd row 8th cell	V
	S31	SA right 4th row 18th cell	A
	S32	SA right 6th row 10th cell	A
	S33	SA left 6th row first cell	V
	S34	SA left 5th row 3rd cell	V
	S35	SA left 4th row 18th cell	A
	S36	SA left 2nd row 2nd cell	V
	S37	SA left 2nd row 16th cell	A
	S38	FC3	A
	S39	FC4	V

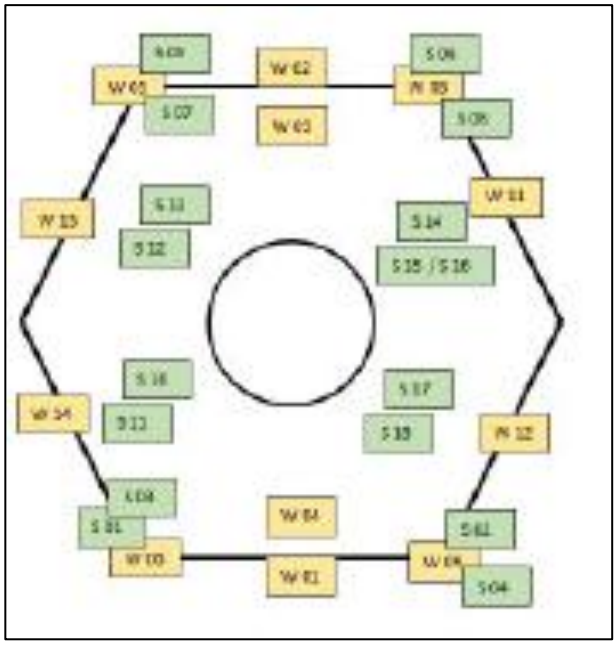


Figure 5-37: Example of a record sheet when performing swab assay, with locations and operators identified (credit: DLR)

Figure 5-38: Example of personnel at work in cleanrooms performing swab assay (credit: TASI (Turin))

After sampling, samples are then taken to the laboratory, where the processing is performed. Each tube containing the buffer is vortex mixed for approximately 5 - 6 seconds. Sonification is also needed.

The heat shock is subsequently performed by water bath at 80 °C ± 2 °C for 15 minutes, as determined by a pilot tube containing a thermometer and making sure that the water bath level is above the level of the liquid content of each tube being heated. After heat shock, the tubes are rapidly cooled for e.g. in ice or cold solution.

Plating is then performed by aseptically pipetting 0,5 mL aliquots of the swab extraction suspension onto the surface of 4 R2A or TSA petri plates, using a total of 2 ML volume.

Incubation at 32 °C is performed with counting of CFU (Colony Forming Units) happening at 24 hrs, 48 hrs and 72 hrs (final count).

It is important to process at least two "lab negative" controls, by moistening the head of a sterile swab in sterile water and process as usual.

Use of wipes.

The process flow for wipe assay is shown in Figure 5-39.

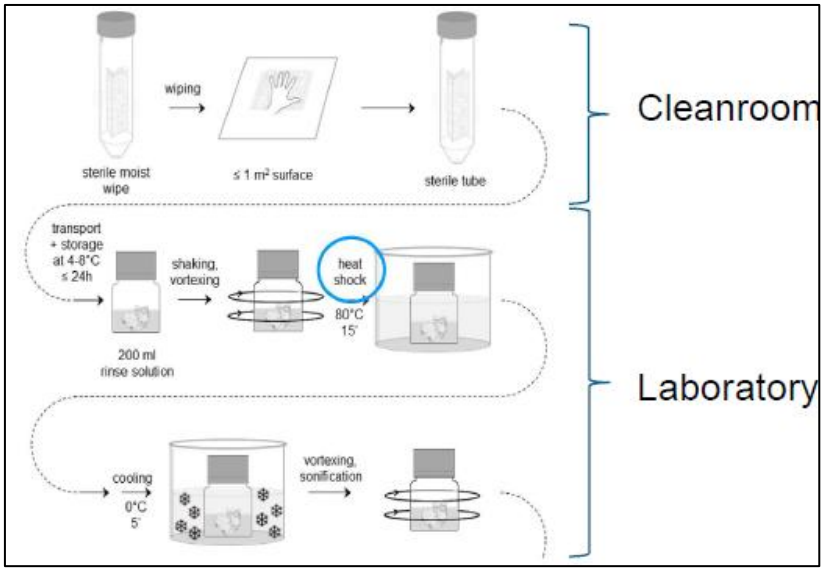


Figure 5-39: Flow chart for standard wipe assay (operations performed in cleanroom and laboratory)

Wipes are used to assay surface areas higher or equal to 1 m2. For the wipe assay procedure, a sufficient number of 15 cm x 15 cm polyester wipes, type TX3211 are pre-wetted in 4mL water, ASTM type IIB, and sterile transport tubes or jars with buffer (20 ml PBS + 0,02 v/v % Tween 80, pH 7,2) to accommodate all samples to be collected, plus controls.

The wipe is placed flat on the sample surface and the entire surface rubbed over, using a firm, steady pressure. The wipe is then re-folded by reversing the direction of the open fold, so the contaminated surface is interior in the new configuration. The wipe is rubbed over the sample area for a total of three times, rotating the direction of motion 90 degrees and 135 degrees, respectively, after each complete sampling of the area, reference Figure 5-40.

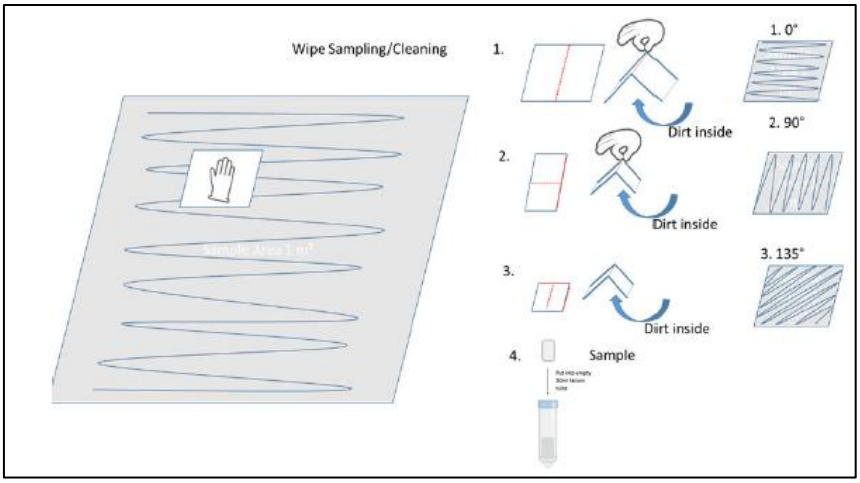


Figure 5-40: Sketch showing the technique for sampling surfaces with swabs

The wipe is then transferred into a sterile transport jar or tube. Samples are transferred to the laboratory, stored at 4 °C – 8 °C and process performed within 24 hours. The type of wipe TX3211 used for ESA spore assay is shown in Figure 5-41.

Figure 5-41: Types of wipes used to perform the ESA spore assay

Examples of wipe assays being performed and how the polyester wipe is kept flat is shown in Figure 5-42.

Figure 5-42: Examples of ESA wipe spore assay

Similarly to swab assays, two people are needed to perform the wipe sampling, with an assistant and the assay taker. The outer pair of sterile gloves is changed by the operator after each wipe assay. Recording and identification of all locations assayed, including pictures of the areas being assayed as it happens) are the same as for swab assays.

A "field negative" is collected every 6 or fewer samples, by placing a sterile 15 cm x 15 cm wipe pre-wetted with 4 mL of water into a sterile transport tube or jar with buffer solution.

Once in the laboratory, wipes are transferred inside a sterile cabinet to a 500 mL flask. 200 mL of sterile rinse solution is the added to each of the flasks containing a wipe. Shaking is generally done by hand for 15 seconds, ultrasonic bath follows for 2 minutes. The flask is then transferred to a heated water bath until 80 °C temperature is reached and maintained for 15 minutes. Temperature is checked by testing a pilot flask containing a thermometer or a thermocouple for the desired time. It is important that the water level bath is maintained above the level of the flask(s). After heating, it is important that the flask is cooled rapidly in a cold bath or in ice.

At this point, it is important to note that there are two possible options: the first one if use a membrane filtration system (i.e. Millipore). This implies the use of one agar plate for each wipe. The second option is performing plating on 13 agar plates (per each wipe), see Figure 5-43.

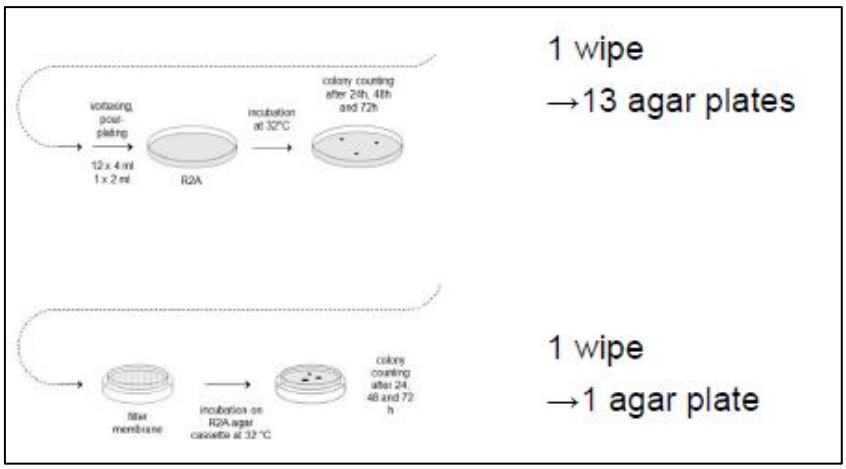


Figure 5-43: Options for plating - ESA standard spore assays with wipes

Millipore membrane filtration system includes the use of a sterile funnel The first step generally consists of pouring and filtering 20 ml of sterile water.

The flask containing the wipe is then shaken vigorously for 15 seconds aseptically. 160 ml volume is poured / filtered into the funnel, followed by 2x rinsing with 100 mL of sterile water

Some pictures about the Milliflex® filtration system are shown below (Figure 5-44 and Figure 5-45).

Figure 5-44: Details of the Milliflex® system (left side), with the mount funnel on top (right)

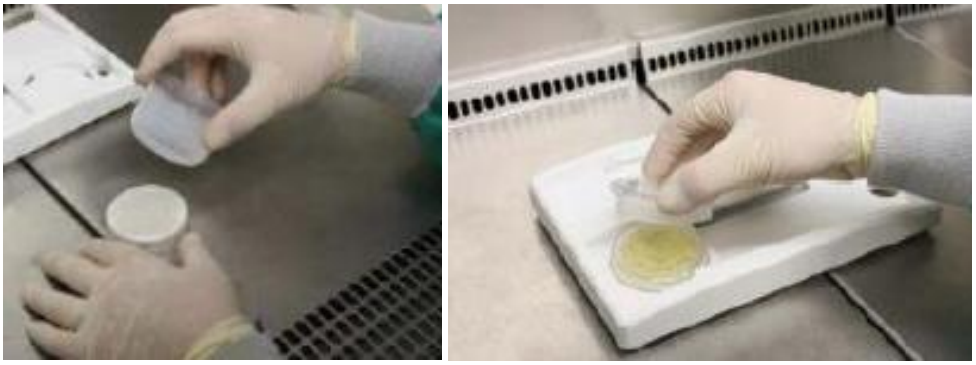


Figure 5-45: Funnel is removed from pump head and membrane (left); the membrane (0,45 μm) is transferred onto R2A or TSA nutrient agar cassette

If a membrane filtration system is not used, 13 sterile R2A plates previously labelled can be used, by pipetting 4mL aliquots of the wipe extraction suspension onto the surface of each plate

Colony counting (for both methods) happens after incubation at 32 °C per 24, 48 and 72 hrs.

It is important to have at least 2 "lab negative" controls, by placing a sterile wipe prewetted with 4 ml water and process as applicable.

The number of samples to evaluate the bioburden for the flight hardware is typically:

- Five swabs for each surface area on flight hardware of 0,1 m^2
- A proportionate number, but at least one swab, for each surface area on flight hardware smaller than 0,1 m^2
- One wipe for each surface area on flight hardware in the size range of 1 m^2
- Two wipes for each individual surface area on flight hardware per 10 m^2

Although the sampling plan is specific to the size and geometry of the sampled hardware, sampling at least 20 % of the surface area is a reasonable guideline. On ExoMars project, the general practice was to assay between 12,5 % and 20 % To reduce the conservatism in the overall bioburden calculations it is better to sample larger surface areas.

To meet the requirements contained in the bioburden control plan for flight systems, it is important to perform bioburden assessments on spacecraft hardware:

- Prior to apply a bioburden reduction procedure (e.g., DHMR – Dry Heat Microbial Reduction)
- 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 can increase the bioburden for the spacecraft hardware (and spare, if applicable);
- As verification assays prior to launch.

Bioburden calculations

The formula to calculate the spores on the area represented by a sample set (assay group) is shown below. With the equation in Figure 5-46, spore densities and total number of spores can be derived by knowing the number of mesophilic, heat-resistant bacteria / spores cultivated in the lab as described above.

$$B = \sum_i \left(N_{Si} + N_{Wi}\right) \Big/ \left[e_S \sum_i p_{Si}\, a_{Si} + e_W \sum_i p_{Wi}\, a_{Wi} \right]$$

$$N(est) = B\, A_0$$

B = estimated burden density for the sample set
A_0 = the area of the hardware represented by the sample set
NSi = number of spores on the ith swab
NWi = number of spores on the ith wipe
aSi = area sampled by the ith swab
aWi = area sampled by the ith wipe
eS and eW are the recovery efficiencies for swabs and wipes, respectively
pSi = pour fraction for the ith swab
pWi = pour fraction for the ith wipe
N(est) = the estimated number of spores on (the surfaces) of the hardware represented by the sample set

Figure 5-46: Bioburden calculation formula

To be noted in the formula the recovery efficiency values for swabs and wipes, eS and eW respectively. These are given values in the equation and are specific for COPAN FLOQSwab 552C nylon flocked swabs and TEXWIPE TX3211 (polyester) wipes used in the ESA spore assay. The recovery efficiency values to be used in the equation are:

- 0,45 for swab assay
- 0,2 for wipe assay

Also, it is important to note that there are given values for the pour fraction, as not all the solution is used/poured in the process. These values are specific and linked to the ESA spore assay method described above. The pour fraction values to be used in the equation are:

- 0,8 for swabs
- 0,25 for wipes

In bioburden assessments, an "assay set" is an important concept to understand. This is defined as a set of assays (wipes, swabs or combination of wipe and swabs) on hardware handled in the same way, post manufacturing cleaning, with the same exposure and cleanliness history and the assays taken in one campaign.

If one or more of the conditions mentioned above is not met, the bioburden formula to calculate total number of spores and densities cannot be applied.

An example of how to calculate bioburden level is presented in the example shown in Figure 5-47.

Set	Assay	Item	m2	Cfu	Sampled area of Set	Represented area for Set
Set #1	w1	2201 Blanket	0.26	0	6.48	25.89
	w2	2112 Blanket	0.26	0		
	w3	2117 Blanket	0.38	2		
	w4	2118 Blanket	0.38	0		
	w5	2127 Blanket	0.34	0		
	w6	2138 Blanket	0.4	1		
	w7	2301 Blanket	0.7	0		
	w8	2140 Blanket	0.4	0		
	w9	2110 Blanket	0.36	0		
	w10	2310 Blanket	0.74	0		
	w11	2107 Blanket	0.36	6		
	w12	2108 Blanket	0.36	2		
	w15	2304 Blanket	0.7	1		
	w16	2332 Blanket	0.8	0		
	s1	2342 Blanket	0.0025	0		
	s2	2342 Blanket	0.0025	0		
	s3	2342 Blanket	0.0025	1		
	s4	2342 Blanket	0.0025	0		
	s5	2342 Blanket	0.0025	0		
	s6	2311 Blanket	0.0025	0		
	s7	2311 Blanket	0.0025	0		
	s8	2351 Blanket	0.0025	0		
	s9	2351 Blanket	0.0025	0		
	s10	2351 Blanket	0.0025	6		
	s11	2413 Blanket	0.0025	0		
	s12	2413 Blanket	0.0025	0		
	s13	2416 Blanket	0.0025	0		
	s14	2416 Blanket	0.0025	0		
	s15	2321 Blanket	0.0025	0		
	s16	2321 Blanket	0.0025	2		
	s17	2321 Blanket	0.0025	0		
	s18	2321 Blanket	0.0025	4		
	s19	2321 Blanket	0.0025	0		

Figure 5-47: MLI bioburden assay - example of assay set calculation

A bioburden campaign has been performed on a Multi-Layer Insulation (MLI). The respective assay set comprises of a combination of swabs and wipes. Figure 5-47 shows pictures of the assays taken as they happened, information about the area of each item assayed, the total sampled area, the number of colony forming units post incubation and the represented area for that specific set are provided. To be noted that about 25 % of the total surface area has been assayed in this specific case (6.48 vs 25.89 m^2).

Applying the formula in Figure 5-46, B = 73,7 spores/m^2, while the total count N = B A_0 = 73,7 x 25,89 = 1909 spores.

In case bioburden assessments cannot be performed, very restrictive assumptions for bioburden densities can be taken. These are also called "specification values" and are estimated based on cleanroom classes and cleanliness environments (see Table 5-7).

Table 5-7 : Bioburden assumptions

Bioburden type	Specific environment	Bioburden value
Average encapsulated spores density (i.e. if no differentiation between electronic and non-electronic piece parts is made)	Non-metallic parts of the spacecraft:	130 spores/cm3
Source specific encapsulated spore density	Electronic piece parts:	3-150 spores/cm3
Other non-metallic materials:		1-30 spores/cm3
Source specific enclosed surface spore density, e.g. a box closed in the specific environment	ISO class 8 cleanroom, highly controlled:	500-5000 spores/m2
ISO class 8 cleanroom, normally controlled:		5000-105 spores/m2
Uncontrolled environment:		105-106 spores/m2
Average surface spore density for cleanroom classes "in operation" (exposed and mated but non-encapsulated)	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 environment:		105 spores/m2
NOTE 1: Manufacturing processes can potentially be used to claim a lower encapsulated bioburden either through high temperatures (see ECSS-Q-ST-70-57 for relevant specifications) or control of manufacturing environment. NOTE 2: Normally controlled: use of gowning equivalent to the specific cleanroom class. NOTE 3: Highly controlled: bioburden control of cleanroom by use of full body coverall, hood, face mask, gloves and boots, restricted access and dedicated cleaning and microbial sampling.		

It is important to note that these values can really penalise a project bioburden budget, especially if used in equipment having large surfaces areas. For e.g., looking at a standard ISO 8 (normally controlled) environment, the spore density assumed in the Table 5-7 is extremely high, if compared with typical bioburden data collected from actual cleanrooms. The take home message here is, specification values can be used only in exceptional cases where it is not possible to assay hardware and when hardware is small, i.e. surface areas values are low. Otherwise, it is recommended to perform as many assays on hardware as possible, rather than assuming too conservative specification values.

5.8.4 Mitigation measures

Certification of labs and personnel

It is important that bioburden assessments are performed by certified microbiology laboratory and personnel.

The certification process at ESA consists of an audit led by the ESA PPO to the laboratory premises where bioburden assessments are foreseen. During the visit, the following can be checked and discussed:

- **Lab quality control documentation**. Expectation is for the lab to have documents or tool in place for managing lab activities, including (as a minimum): definition of roles and responsibilities for lab personnel / managers, how data are recorded and retained in the system, activities needed to have a re-certification, list of equipment used and re-calibration, user manual for all the equipment in the lab, health and safety aspects.
- **Operating procedures**. Expectation is for the lab to have a procedural document detailing the different methods for performing bioburden assessments, in line with relevant ESA documentation, i.e. ECSS-Q-ST-70-55. This includes a step-by-step procedure from the sterilisation and preparation of kits in the lab, to how sampling is taken in cleanrooms and flight hardware, activities recorded and photographed, transported back to the lab, stored, and processed. Measures to avoid false positives can also be included in this procedure, such as lab attire when performing the processing, field lab negatives, description of the stations/laminar flow cabinets used, sterilisation of bottles, jars or any consumable before being introduced inside the working area, etc.
- **Laboratory tour**. During the tour, the ESA PPO can check the suitability of the lab environment, its set-up, equipment used, as well as the adequacy of lab attire.
- **Demonstration of competencies**. Expectation is for ESA PPO to witness lab personnel in simulating (as much as possible) of the end-to-end bioburden assessment process. This includes witnessing swab and wipe assays techniques, best practices adopted for recording information, processing the samples, heat shock, pipetting and pouring on agar plates etc. On a case-by-case basis, it can be requested to verify the ability of lab personnel to perform specific tasks, such as serial dilution.

At the end of the certification process, a certificate can be released by the ESA PPO, with a statement certifying the lab and a list with personnel qualified to perform specific project biological assays on cleanrooms and flight hardware. The certification has typically a duration of 1 year. However, on a case-by-case basis, ESA can delegate the re-certification activities to the laboratory managers, providing a report with the outcome of re-certification and a summary of the demonstration of competence is sent to ESA PPO for endorsement or information.

Compatibility of hardware with assay process

Biological assays prescribe the use of sterile water to rinse swabs head or wipes. Use of water can be detrimental for flight hardware, mainly because:

1. It can create short circuits if used on electronic components or equipment.
2. It can create oxidation and corrosion if used on unfavourable bimetallic contacts, for e.g. on a couple made of Aluminium and stainless steel.
3. It can damage coatings, such as Anti Reflecting (AR) used on optics.

In order to prevent damage of hardware caused by the assay process, it is important that the equipment or subsystem or the system contractor indicates in their Planetary Protection plan the areas on the hardware where a biological assay is forbidden due to potential damage. A sketch or drawing of the

hardware, clearly showing "NO ASSAY" zones, is the preferred method to identify potential compatibility issues with water. Having a visual representation of those areas is important considering that lab personnel that is not familiar with technical descriptions of flight hardware.

On a case-by-case basis, use of alcohol IPA 99 % can be permitted instead of sterile water, providing the change is traced and recorded for example via a request for waiver (RFW). Reason to have a RFW is that recovery efficiency of swabs or wipes can change from their values discussed in this Section 5.8.4 if IPA 99 % is used.

Importance of following step-by-step procedures

It is important that prescribed methods, tools, consumables as per ECSS-Q-ST-70-55 are used, as these have a direct effect on the recovery efficiency and pour fraction and, as a consequence, on the overall mathematical equation (see Figure 5-46) used to calculate bioburden density and total count. If different swabs or wipes materials or methods are used, it is important that the lab provider justifies their choice with a sound statistical basis.

Coordination with Planetary Protection and contractors

It is advisable that the Planetary Protection function in the project helps & supervises biological assay campaigns. Laboratory personnel are not necessarily involved in project activities or key reviews (i.e. PDR, CDR). As a consequence, important details needed for the assay can be missed, such as information about minimum surface area to be assayed, compatibility of materials with assays, specific coating used on cameras, or use of electronic units, i.e. on Printed Circuits Boards (PCBs). Engineering support provided by PP discipline to the laboratory is therefore highly encouraged

Coordination between contractor and the lab performing the assay is essential in preparation for the campaign and to ultimately stay within the 24 hrs elapse time between assay campaign and processing back in the lab. The following points can be considered and recorded in a "check-list" at the time of coordination between lab and contractor:

- Check availability of a freezer at contractor premises. This is needed for storing ice packs used to keep samples cooled during storage and transport to the lab
- Make sure the laboratory is familiar with the hardware to be assayed, knows the criticalities in terms of areas compatible with assay procedure, and "NO ASSAY" areas are identified and understood
- Make sure the laboratory knows the manufacturing stage and conditions, especially in terms of accessibility. Some areas are difficult to access, and special care can be taken to minimise the risk of physically damaging flight hardware due to lack of accessibility
- Get confirmation from the contractor that flight hardware is available at the time the assay is scheduled, as personnel cannot work on it during the slot allocated for the assay. This point is extremely important, as programmatic, time/schedule constrains are always part of space industry. If this is seen as an issue, it is important to manage expectations with the AIT teams or chose a shift (i.e. night) where project schedule is less affected. This is especially relevant during final integration and testing stages or at equipment level, when at least 2 assay campaigns per equipment can be scheduled
- Check that the assay is performed in one campaign on hardware handled in the same way, post manufacturing cleaning, with the same exposure and cleanliness history (assay set concept)
- Check whether proxy samples or mock ups have been considered and need assaying, for e.g. after TVAC for estimation of internal surfaces recontamination is a driver for example

Heat shock bath

Water or ice used for performing the heat shock can perhaps be considered one of the main sources of contamination during processing of samples. When performing the heat shock step, it is important that the jar is dried and clean with IPA 70 % to avoid any water residue before the next step is performed. It is equally important to have a plan in place to frequently change the water in the bath.

5.9 Bioburden reduction

5.9.1 Examples of bioburden reduction requirements

Typical bioburden reduction requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.4.

Clause 5.4.4 Bioburden reduction

a. *Dry heat microbial reduction shall be performed in compliance with requirements from clause 5 of ECSS-Q-ST-70-57.*

b. *Hydrogen peroxide microbial reduction shall be performed in compliance with requirements from clause 5 of ECSS-Q-ST-70-56.*

c. *Use of other bioburden reduction procedures shall be approved by the PPAA.*

d. *Evaluation of material and hardware compatibility with bioburden reduction procedures shall be performed in compliance with requirements from clause 5 of ECSS-Q-ST-70-53.*

5.9.2 Rationale for the requirement

Bioburden reduction is defined as the process or processes used to reduce the viable microbial population of an item to acceptable limits. That differs from sterilisation, which is used to render surfaces free from viable micro-organisms. Although sterilisation can be achieved, it won't be sustainable overtime. In Planetary Protection, the term sterilisation is often used to indicate a bioburden reduction.

The rationale for having such requirements is to define protocols and procedures to consistently determine microbial reduction levels on space hardware.

Bioburden reduction processes need to be carefully designed, qualified and monitored to keep probability of contamination of flight hardware very low. There are different methodologies in Planetary Protection that can be used, including alcohol wiping with IPA 70 %. This Section 5.9.2 mainly focuses on the most common:

1. Dry Heat Microbial Reduction (DHMR), used with temperature over 110 °C, for a given duration and controlling relative humidity.
2. Hydrogen peroxide (gas plasma).

Compatibility of materials with bioburden reduction processes is a critical aspect that needs high consideration in projects and when choosing one method over another. Materials used to build space hardware, when subject to bioburden reduction, can deteriorate their performances, for e.g. by decreasing mechanical properties, oxidation or even developing of cracks, if measures are not taken at design and testing level.

Some general examples of potential detrimental effects on materials using DHMR, hydrogen peroxide and radiation are shown in Figure 5-48.

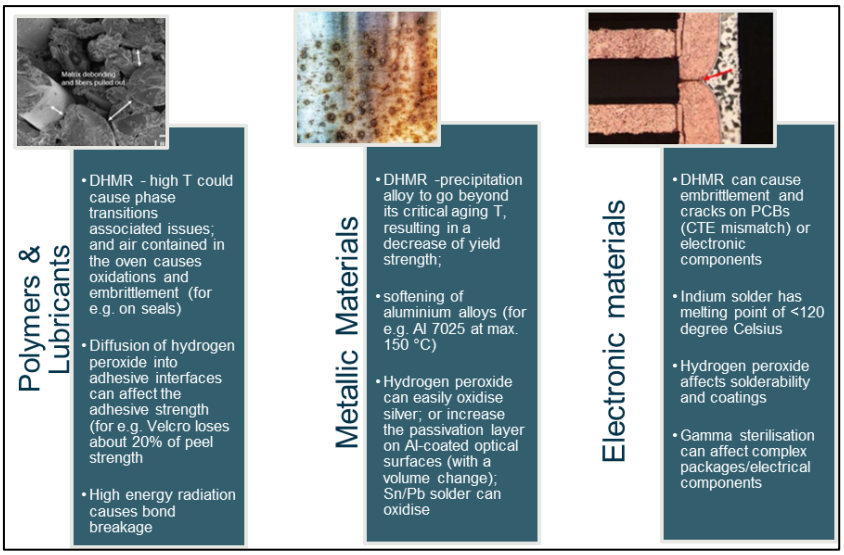


Figure 5-48: Notional diagram of material classes and potentail detrimental effects caused by bioburden reduction processes

5.9.3 Methods to assess compliance

Dry Heat Microbial Reduction (DHMR)

DHMR is a process used for the reduction of microbial contamination of flight hardware using heat.

Such reduction can involve exposed, mated surfaces and encapsulated volumes in materials. It is important to recall some definitions to fully understand the effect of a bulk bioburden reduction such as DHMR.

Exposed surfaces are those internal and external on hardware, free for gas exchange. This is an important concept in Planetary Protection and can be explained with a practical example and considering the exposed surface areas of a laptop.

Figure 5-49: Notional example of a laptop, with breakdown of all its component, to explain the concept of exposed surface areas

In the example in Figure 5-49, the external surfaces are represented by the top, bottom and side panels, the keyboard and the screen.

The internal surfaces are inside the laptop; these are the bottom side of the keyboard, the inside of the screen, the battery, the circuit boards, the fan and the hard drive. All the internal surfaces are available for gas exchange (in this specific example, any internal contaminant is actually pushed out by the action

of the fan). As such, both internal and external surfaces of a laptop are considered exposed surfaces and considered for bioburden calculations.

Similarly, for spacecrafts, it is important to consider all internal surfaces. Figure 5-50 and Figure 5-51 show examples of internal (but still accountable) surfaces, such the ones in honeycomb cells used in carbon fibres reinforced materials (CFRPs), and outer or inner plies surfaces of multi-layer insulation (MLI). The internal of CFRP and MLI can actually account for quite big surface areas, as such they need attention in the initial budget allocation.

Details of a composite material CFRP: substrates for Solar array panels (top Figure), aluminium honeycomb core being cut in the lab from the carbon fibres skins and rinsed with PBS solutions to assess biological contamination on internal surfaces (bottom Figure). Credit: Airbus UK/Thales Alenia Space Italy (Turin).

Figure 5-50: Example of a CFRP used in space hardware

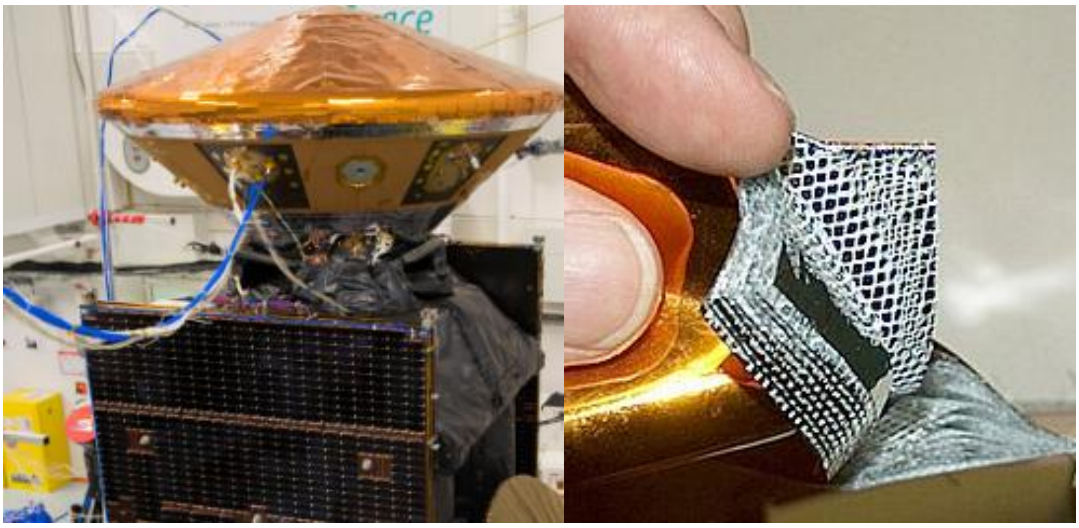


Figure 5-51: Multi Layer Insulation (MLI) used on Entry Descend Module (ExoMars 2016) – left. (Deatils of the MLI layers and internal surfaces to be considered for Planetary Protection purposes on the right)

Mated surfaces are those joined by fasteners rather than adhesives.

Encapsulated bioburden is buried inside bulk non-metallic materials, i.e. it is not free for gas exchange. Examples include bioburden inside paints, coatings, adhesives, inserts, ablative materials.

Before applying a DHMR process, it is important that:

1. The hardware meets specific cleanliness levels (particulate and molecular). These are defined for example in IEST-STD-CC1246, level ≤ 300A.
2. In case of hardware, which is expected to release high amount of volatiles, a pre-conditioning with heat before DHMR can be performed to minimise contamination.
3. Bioburden assays are performed as described in Section 5.8 and per ECSS-Q-ST-70-55.
4. Any package (if present) is removed or its compatibility with DHMR assessed.
5. Label to identify hardware being subject to DHMR.
6. Compatibility of DHMR with flight hardware is demonstrated.

For compatibility purposes (point 6), while assessment of individual materials for e.g. by reviewing declared material and processes lists is good practice to initiate scrutiny, compatibility can be fully verified only at equipment or system level. A typical example to explain the importance of performing assessment beyond single material level is considering the effect of high temperature during DHMR on individual metals, such as stainless-steel, aluminium and titanium alloys. Each of these metals can easily withstand temperature of 110 °C - 125 °C, which are typically used for DHMR. However, if stainless steel, aluminium and titanium alloys are joined together in an assembly, with complex geometry and thin sections (see Figure 5-52), high temperature can become an issue, as it can create creep effects and distortion.

Figure 5-52: Detail of the bogies electro-mechanical actuator and complex geometry of wheels of the ExoMars Rosalind Franklin Rover. Credit: Airbus UK

In those cases, special attention needs to be paid on material combinations, due to potentially significant differences in the coefficient of thermal expansion (CTE) between two or more components. When designing space hardware systems, it is important to select materials with CTE mismatch kept to the minimum.

Similar considerations as for metals, are valid when choosing polymers or adhesives for e.g. structural bonding, carbon fibres reinforced plastic (CFRP) or surface mounting assembly of printed circuit boards (PCBs).

For adhesives, or foams used in CFRP, CTE mismatches can be even more exacerbated by the effect of outgassing (due to presence of vacuum), leading to higher CTE than originally expected and declared in datasheets.

Dis-bonding, cracks on PCB materials or metals are not uncommon during qualification of hardware, especially when environmental tests require survival within large temperature ranges. For a Mars mission, certain equipment need qualification over hundreds of (non-operational) cycles between -130°C and + 90°C, plus evidence of withstanding sterilisation temperature used in DHMR, i.e. 125°C. Under those extreme temperature conditions, materials are subject to high thermal stresses, i.e. they contract when going below 0°C and expand when going to high temperature. As such, it is essential to choose adequate material pairs (i.e. with similar CTE) and "design for Planetary Protection". The effect of high temperature that can be used in sterilisation processes via DHMR on electronics, i.e. PCBs, has been preliminary studied by ESA, and it is believed that the high temperature used during DHMR can start damaging materials (without being immediately visible for e.g. by inspection) by increasing grain size on solders, with propagation of cracks happening during subsequent thermal cycling as applicable.

DHMR process parameters.

The duration of a DHMR process is a function of humidity. In fact, desiccation is a main stressor for microbes. Lower the relative humidity, less is the time needed to reach the same microbial **Log** reduction.

For definition of ***Dry humidity, Ambient humidity and Uncontrolled humidity*** refer to Section 3.

Humidity level can be achieved by using dry gas (filtered to 0,2 µm) or vacuum (in fact, a DHMR can be combined with a vacuum thermal bake-out).

The starting time for DHMR is driven by the coldest location as measured on the hardware and humidity levels inside the chambers. When these values are met (as applicable) the process can start. The coldest location is determined on the product item either during the cycle or on a test model in the product specific cycle development

A performance qualification of the system used for bioburden reduction can be done to demonstrate that the system performs in accordance with the bioburden reduction procedure and cycle requirements

Minimum temperature = 110° C

Maximum temperature = 200° C

D-value = time needed to inactivate 90 % of a population of the test microorganism under stated conditions. Equations for temperature dependent D-values (for **Log 1 to 3** only) for surface bioburden are indicated in Figure 5-53.

Temperature	Dry Humidity	Ambient Humidity
T ≤ 140 °C	$D(\text{min}) = 30 \times 10^{\frac{(125-T)}{21}}$ [5-1]	$D(\text{min}) = 5{,}79 \times 10^{\frac{(140-T)}{18}}$ [5-3]
T > 140 °C	$D(\text{min}) = 5{,}79 \times 10^{\frac{(140-T)}{(23\times T)/140}}$ [5-2]	$D(\text{min}) = 5{,}79 \times 10^{\frac{(140-T)}{(23\times T)/140}}$ [5-2]

Figure 5-53: Extract from ECSS-Q-ST-70-57 for Log 1 and Log 3 surface bioburden D-values

For mated bioburden, **multiply duration by 2.** Rationale: inhibition of moisture loss in these locations protects microbes from desiccation.

For encapsulated bioburden, **multiply duration by 10.**

Figure 5-54 (from ECSS-Q-ST-70-57) gives a summary of duration, temperature and humidity conditions for the temperature range applicable for DHMR process.

Temperature	dry surface	ambient surface	dry mated	ambient mated	uncontrolled humidity (surface, mated) and encapsulated
T (°C)	D_{value} (min)	D_{value} (min)	D_{value} (min)	D_{value} (min)	D_{value} (min)
110	155,4	268,7	310,8	537,5	1533,8
111	139,2	236,5	278,5	473,0	1392,5
112	124,8	208,1	249,6	416,2	1247,9
113	111,8	183,1	223,7	366,2	1118,3
114	100,2	161,1	200,4	322,2	1002,1
115	89,8	141,8	179,6	283,5	898,1
116	80,5	124,7	161,0	249,5	804,8
117	72,1	109,8	144,2	219,5	721,2
118	64,6	96,6	129,3	193,2	646,3
119	57,9	85,0	115,8	170,0	579,2
120	51,9	74,8	103,8	149,6	519,1
121	46,5	65,8	93,0	131,6	465,2
122	41,7	57,9	83,4	115,8	416,8
123	37,4	50,9	74,7	101,9	373,6
124	33,5	44,8	67,0	89,7	334,8
125	30,0	39,4	60,0	78,9	300,0
130	17,3	20,8	34,7	41,6	173,4
135	10,0	11,0	20,0	22,0	100,2
140	5,8	5,8	11,6	11,6	57,9
145	3,6	3,6	7,1	7,1	35,7
150	2,3	3,6	4,5	4,5	22,7
160	1,0	1,0	2,0	2,0	10,0
170	0,5	0,5	1,0	1,0	4,88
180	0,26	0,26	0,5	0,5	2,57
190	0,14	0,14	0,3	0,3	1,45
200	0,09	0,09	0,2	0,2	0,86

Figure 5-54: Extract from ECSS-Q-ST-70-57: D-values for 2 to 3 order of magnitude reduction

There is a substantial difference in the time needed between 3 and 4 orders of magnitude reduction Rationale: sub-populations of typical cleanroom bioburden are more resistant to heat than the average population

For **Log 4** or higher reductions, humidity is not needed, because of the extended time period at temperature is able to lower humidity to needed levels.

Effective D-values are used for bioburden reduction of ≥ 4 orders of magnitudes and are calculated as per equations in Figure 5-55 (extract from ECSS-Q-ST-70-57):

- One effective D-value is 4 orders of magnitude reduction.

- Two effective D-value is 5 orders of magnitude reduction.
- Three effective D-value is 6 orders of magnitude reduction.

$$D(hours) = 10^{-3,5991+\frac{2049,0923}{(T+273)}}$$

$$D(hours) = 10^{-19,1595+\frac{8320,082}{(T+273)}}$$

Figure 5-55: Temperature dependent effective D-value in hours for 4 to 6 order of magnitude surface bioburden reduction

For **Log 5** reduction, multiply effective D-value by 2. For **Log 6** reduction, multiply effective D-value by 3.

NOTE A maximum of **Log 4** reduction can be credited for T ≤ 125 °C. The full 5 to 6 order of magnitude reduction can only be credited for T > 125 °C.

Figure 5-56 is taken from ECSS-Q-ST-70-57 and show the effective D-value temperature and time parameters for **Log 4** to **Log 6** surface reduction.

Temperature T (°C)	Effective D_{value} (hours)
110	56,4
111	54,6
112	52,9
113	51,2
114	49,6
115	48,1
116	46,6
117	45,2
118	43,8
119	42,5
120	41,2
121	40,0
122	38,8
123	37,6
124	36,5
125	35,4
130	30,6
135	17,1
140	9,7
145	5,6
150	3,2
160	1,1
170	0,42
180	0,16
190	0,065
200	0,027

Figure 5-56: Effective D-value for 4 to 6 order of magnitude surface reduction (see ECSS-Q-ST-70-57)

Examples on how to determine DHMR parameters

Scenario 1: For a mission to Mars, a CFRP material structure used on the lander has an initial bioburden density of 300,000 spores/m^2. The project bioburden budget planned for the lander to be delivered to customer to 300 spores/m^2. The lander needs therefore a **Log 3** reduction. Also, the lander has only been qualified (during qualification model – QM activities) to 125 °C. Which DHMR parameter does the project use for reaching the desired **Log 3** reduction?

Answer 1: the structure of a lander has usually a very high surface area. This is due to the material used, CFRP, which is made of a honeycomb core (aluminium) and carbon fibres skins. The honeycomb is the greater contributor to the total surface area.

The objective to is get a lo **Log 3** reduction on all surfaces of the CFRP lander (exposed, mated and encapsulated). Encapsulated bioburden is considered by project due to the potential risk of non-nominal landing on Mars surface, as any bioburden can be liberated as a result of impacting.

Looking at Figure 5-54, within the table, the last column gives the duration in minutes of D value for surface, mated and encapsulated bioburden. At 125 °C, this time is 300 minutes.

As such, in order to get a **Log 3** reduction up to the encapsulated level, the DHMR process duration needs to be: **300 minutes x 3 = 900 minutes.**

Scenario 2: For a landing mission to Mars, the Rover's wheels are made of metals. The initial bioburden density is 3000 spores/m^2. To meet the initial bioburden budget allocation of 30 spores/m^2 given to the wheels, a **Log 2** reduction is needed. The hardware has only been qualified up to 110 °C, and project can only guarantee a humidity control of less 12 g/m^3 inside the chamber. What DHMR parameters can project use to decrease bioburden densities to 30 spores/m^2 ?

Answer 2: In this case the objective of DHMR is to achieve a **Log 2** bioburden reduction on surface and mated surfaces. There are not concerns on encapsulated bioburden in this case, as the assembly is entirely made of metal. Humidity can only be controlled and verified to ≤12 g/m^3, which is the conditions named as "ambient".

Looking at Figure 5-54, within the table, the D value for "ambient mated" at 110 °C is 537,5 minutes. Given the target of reaching a **Log 2** reduction on mated surfaces, this duration is multiplied by 2.

As such, the DHMR duration for ambient mated condition to reach a log 2 reduction is : 537,5 x 2 = 1075 minutes

Scenario 3: For a landing mission to Mars, a structure has been biological assayed and the total amount of spores was calculated to be 5 000 000 spores. The initial budget allocation for the structure at delivery to customers was 50 total spores. Project therefore needs a **Log 5** reduction to meet the bioburden target. The hardware has been qualified up to 130°C. What DHMR parameters can project use to decrease the total number of spores to 50 total?

Answer 3: For **Log** reduction higher than 3, project needs to look at D-effective values. From Figure 5-55 and Figure 5-56, 30,6 hrs are needed at 130 °C for a surface reduction to reach a **Log 4**.

Duration of **Log 5** DHMR reduction at 130 °C is therefore:

Surface bioburden = 2 x 30,6 hours = 61,2 hours

Mated bio bioburden = 2 x 61,2 hours = 122,4 hours

Encapsulated bioburden = 10 x 61,2 hours = 612 hours

Vapor Hydrogen Peroxide (VHP)

VHP is a process used for the reduction of microbial contamination of flight hardware using hydrogen peroxide vapour. VHP is a surface bioburden reduction methodology, i.e. it does not sterilise bulk materials like it happens with DHMR. The reduction of microbial contamination involves exposed to vapours surfaces, and it can happen in controlled ambient or vacuum environments.

Before applying a VHP process, it is important that:

- The hardware meets specific cleanliness levels (particulate and molecular). These are defined for example in IEST-STD-CC1246, level ≤ 300A.
- Bioburden assays are performed as described in Section 5.8 and per ECSS-Q-ST-70-55.

- Any package (if present) is removed or its compatibility with VHP assessed.
- Label to identify hardware being subject to DHMR.
- Compatibility of VHP with flight hardware is demonstrated.

For **compatibility of materials** with VHP**:**

Organics. Epoxy resins that are cross-linked with a larger number of amino-curing agents can degrade, as well as materials with S-S linkages due to oxidation (e.g. sulphur vulcanised rubbers). In addition, the presence of hydrogen peroxide during the sterilization process can lead to surface oxidation, embrittlement and increase of seal hardness, or paint chipping. Diffusion of hydrogen peroxide into adhesive interfaces can affect the adhesive strength or interact with filler particles (e.g. silver) within the matrix, reducing electrically or thermally conductive coatings performances.

NOTE With Velcro, there can be a loss of 20 % of peel strength, as such margins are recommended.

Metals. Silver can be easily oxidised to Ag_2O by H_2O_2. Pinholes in optical coatings with underlying silver layers or coatings of wires underneath insulation can be effected by H_2O_2 diffusion or penetration. The H_2O_2 sterilization environment can increase the Al_2O_3 passivation layer on Al-coated optical surfaces. This is accompanied by a volume change and can be critical where geometry is crucial, e.g. for grating applications. Corrosion resistant alloys need attention and further investigations, to evaluate their resistance against H_2O_2 environment. Sn/Pb solders: Lead can oxidise if exposed (normally behind conformal coating). Black anodization layers can be damaged during hydrogen peroxide sterilization, in particular if organic dyes are used.

Lubricant. The oxidizing environment can result in conversion of sulphide-based solid lubricants to the corresponding oxides ($WS_2 \rightarrow WO_2$, $MoS_2 \rightarrow MoO_2$), leading to increasing friction in mechanisms. In addition, the sulphides can react with hydrogen peroxide to sulphuric or sulphurous acid that can further damage materials.

VHP for controlled ambient environments

A **Log** reduction between **2** and **6** is expected. The VHP concentration in a controlled ambient environment is typically set to ≥ 1,1 mg/L, with a D-value for surface bioburden reduction of 200 (mg/L)sec. That means, in controlled ambient environments, a **Log 2** to **6** surface reduction can be achieved by multiplying 200 (mg/L)sec by a factor of 2 to 6.

A procedure for overkill can be used under controlled ambient conditions. In this case, the hydrogen peroxide concentration is typically set between 6 mg/L to 8,6 mg/L.

VHP for controlled vacuum environments

A **Log** reduction between **2** and **6** is expected. The VHP concentration in controlled vacuum environments is typically set between 0,5 and 1,1 mg/L, with a D-value for surface bioburden reduction of 200 (mg/L)sec. That means, in controlled vacuum environments, a **Log 2** to 6 surface reduction can be achieved by multiplying 200 (mg/L)sec by a factor of 2 to 6.

Good practice in VHP is the use of biological indicators (BIs). These are viable microorganisms which provide a defined resistance to VHP. Typical commercially available BIs for VHP can be spores of *Bacillus atrophaeus* ATCC 9372 (SGM Biotech, USA) and *Geobacillus stearothermophilus* ATCC 7953 (Steris).

BIs can be placed in locations where it is difficult to achieve the specified bioburden reduction level. Sensor to measure the VHP concentration can be used in conjunction with BIs. The time for starting the time-integration of the process can be reached when the minimum concentration specified by requirements is met.

Qualification is an important phase and typically includes physical and microbiological performances of the process. It is important that the system is equipped with instrumentation to monitor, control and record:

1. Temperature.
2. Time.
3. Pressure and airflow.
4. Humidity.
5. VHP concentration.

5.9.4 Mitigation measures

For DHMR process, there are some common misconceptions that is important to mention, as measures to prevent errors when applying sterilisation processes.

It is important that the values and terminologies related to humidity are fully understood, see Section 3. In particular, "ambient humidity" does not refer to the humidity at the intended facilities. As per definition in ECSS-Q-ST-70-57, this is defined as an absolute humidity of ≤ 12 g/m^3, which is equivalent to 70 % relative humidity at 0°C and 1000 hPa pressure

In addition, separate DHMR cycles cannot simply be summed up. For example: a **Log 3** reduction followed by a (later) **Log 3** reduction ≠ **Log 6** reduction

Assaying of flight hardware takes place just before DHMR (or at point of last access). No assaying is performed post-DHMR. A bioburden reduction is assumed according to the DHMR parameters used.

If DHMR process is applied early in the hardware build process (e.g., at part or sub-assembly stage), it is important to understand that, unless either completely aseptic assembly, or isolation of those items by bio-barriers are employed from that point forward, recontamination can occur.

Compatibility of hardware with sterilisation processes (reference ECSS-Q-ST-70-53) is an essential part during Planetary Protection reviews. The effects can be of two types:

- Direct effects, with changes of intrinsic material properties that cannot observed immediately after sterilisation but can be manifested over longer duration.
- Indirect effects, these are secondary interactions (e.g., molecular contamination, bondage breakage due to CTE) and are caused by interaction with a non-process parameter after application of sterilisation process (e.g., post degradation due to interaction of oxygen from air with "active" centres generated during sterilisation). These can also be manifested over longer duration.

The measure to avoid any type of direct or indirect damage on hardware consist in following a correct qualification approach, as mentioned in Section 5.9.2.

Figure 5-57 (extract from ECSS-Q-ST-70-58) shows the steps to be considered when designing bioburden reduction processes.

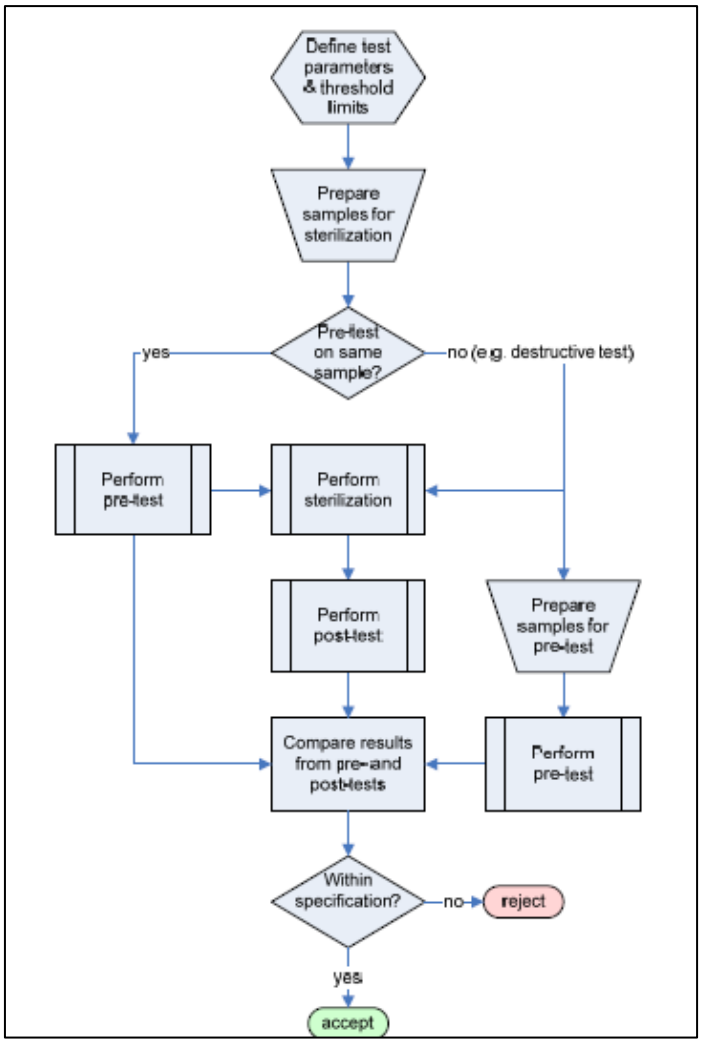


Figure 5-57: Test procedure flow diagram for sterilisation (see ECSS-Q-ST-70-58)

Qualification of hardware starts from materials and components level up to higher assembly level

Compatibility on material level does not necessarily guarantee that it performs to its requirements in an assembly (e.g., CTE mismatch). It is important that a sterilisation process is included in the overall EQM/QM hardware qualification test sequence. Since hardware sterilisation occurs prior to launch, and exposure of hardware to further mission environments occurs afterwards, qualification can be included as a first step in the EQM/QM model testing, prior to environmental testing (e.g. ,Vibration Test, Thermal Vacuum Testing).

5.10 Biodiversity assessment

5.10.1 Examples of biodiversity requirements

Typical biodiversity requirements for Planetary Protection can be found in ECSS-U-ST-20C clause 5.4.

Clause 5.4.3

a. *Biodiversity assessment on flight hardware and bioburden-controlled environment(s) shall be performed using procedures D.3-D.6 for Swab assays and procedures E.3-E.6 for Wipe assays as per Annex D and Annex E of ECSS-Q-ST-70-55.*

5.10.2 Rationale for the requirement

ESA spore assay per ECSS-Q-ST-70-55 is currently the only method used to verify and certify Planetary Protection requirements in Europe This method is able to enumerate aerobic microorganisms that survive a heat shock of 80 °C for 15 minutes (hereinafter "spores") and form visible colonies after 72 hours of incubation at 32 °C on TSA (Tryptic-Soy-Agar). Pre-launch Planetary Protection certifications as applicable (i.e. **Category IV**, landers to Mars) are granted if space missions can demonstrate that the number of spores present on flight hardware do not exceed the acceptable levels.

However, this method is not representative of all biodiversity in cleanrooms and space hardware. A large amount of viable but not cultivable (VBNC) organisms remain undetected by current standard assays. It is estimated that less than 0,1 % of total organisms on a spacecraft can be identified with such method (Ghosh et al, 2010). Culture-based techniques are therefore unable to identify the full range of "problematic" organisms for Planetary Protection, i.e. those capable to survive the journey to outer space, replicate and contaminate celestial bodies.

Reporting of zero CFU (colony forming units) on an agar plate following ECSS-Q-ST-70-55, does not indicate absence of concerning organisms for contamination (i.e. "lack of evidence is not evidence of lack").

Additional culture-based assays for cultivation of oligotrophic, alkaliphilic, vegetative, anaerobic microorganisms and fungi, can be used for Planetary Protection related projects at ESA, currently for biological knowledge (not for verification of bioburden).

Since 2003 ESA is collecting data on bioburden and biodiversity in different spacecraft assembly cleanrooms and from different spacecraft. From 2011 to 2021, 33 Planetary Protection campaigns took place in spacecraft assembly cleanrooms operated by ESA member states, companies and other former collaborators (e.g. Roscosmos) - mainly in preparation for ESA's ExoMars mission. Beside standard cultivation techniques (e.g. the ECSS standard spore assay) also alternative cultivation assays and since campaign 17 in 2016 also next generation sequencing of 16S rRNA gene amplicons was carried out. However, despite this method being cost effective and having standard bioinformatics workflows, there are several drawbacks, such as primer bias (incomplete coverage and estimation of diversity) and limited taxonomic resolution, which makes assessment related to microbes' survivability unreliable.

ESA Planetary Protection function is now introducing systematically nucleic acid sequencing-based approaches (i.e. culture independent), such as metagenomics. These are widely used in different applications, such as in the pharmaceutical, medical and food industries. If properly tailored for space needs, metagenomic methods can provide information on whether species in the investigated microbial community have the genomic potential to survive outer space stressors' (i.e. radiation, presence of salts, low temperature, desiccation). As a consequence, this technology can help assess survivability of organisms even beyond pre-launch activities, and considering biocidal effects of outer space, with direct influence on spacecrafts' design and operations. Developing metagenomic technologies, can trigger a re-thinking of PP approaches, as information can be used to design missions such that contamination risk is acceptable by decreasing efforts in sterilisation and Planetary Protection measures pre-launch.

The overall objective of this requirement is to gather biological knowledge and biodiversity information in cleanrooms.

The complexity of future space missions calls for expanding current Planetary Protection approaches, including tools and methods used to measure biological contamination. Research and technology developments in the field of molecular biology are considered paramount for Planetary Protection. These techniques provide key information to assess contamination risks, in the effort to ensure that target bodies are kept as pristine as possible during the course of astrobiological exploration (forward contamination), and to control and safeguard crew health, general public, and Earth's biosphere (backward contamination).

In the next few years, alternative techniques and methods based on DNA-extraction are planned to be introduced at ESA within Planetary Protection requirements. These are currently under development and can be tailored for space use to feed risk-informed decision frameworks, by providing information on microbes' survivability and, in turn, provide clues on the concept of harmful contamination in outer space.

ESA have already started this process and heavily investing in shotgun metagenomic as part of the different assay campaigns in European cleanrooms, i.e. started the implementation of the Technology 2040 vision, which includes Planetary Protection as supporting technology (ref. ESA - Technology 2040).

5.10.3 Methods to assess compliance

ESA wipe assay for cultivation of oligotrophic, alkaliphilic, vegetative, anaerobic microorganisms and fungi, and molecular analysis.

A sufficient number of sterile (and for molecular analysis DNA free) 23 cm x 23 cm bonded polyester cleanroom wipes are prepared. For aerobic cultivation (oligotrophic, alkaliphilic, vegetative, Fungi) premoistened wipes (with 15 ml water) are autoclaved, and for anaerobic cultivation the wipes are autoclaved without water. For molecular analysis, the wipes are first baked at 170 °C for 24 hours, then premoistened with 15 ml DNA free water and autoclaved.

Sterile gloves for aerobic and anaerobic cultivation sampling, and DNA free gloves for molecular sampling are used.

To take a sample, the wipe is placed flat on the surface and rubbed over the entire surface using a firm, steady pressure. The wipe is refolded by reversing the direction of the open fold so that the contaminated surface is interior in the new configuration. The same sample area is rubbed with the wipe a total of three times, rotating the direction of motion first 90 degrees and then 135 degrees.

Lastly, the wipe is placed into a sterile transport tube. The wipes for aerobic cultivation is stored at +4 °C for maximum of 24 hours and then processed in laboratory. Anaerobic samples are stored at +4 °C for 1 week, and the molecular samples at - 20 °C for 6 days.

For all sample types (aerobic and anaerobic cultivation, molecular analysis) field negative control samples were collected. The control samples are collected by removing a sterile sampling wipe from its tube, opening the wipe and touching it with gloves, and placing it back to the tube. In laboratory, also extraction blank samples were used to control the sterility of reagents and equipment. Controls were processed and analysed in the same way as samples. Additionally, for molecular analysis DNA extraction kit negative control and PCR negative control samples are used, along with the cleanroom samples to control the purity of kit and PCR reagents.

Cultivation assay

Extraction for cultivation

For aerobic cultivation, the wipes are transferred into a 500 ml wide neck bottles prefilled with sterile 1x PBST Buffer. Samples and controls taken from the same room are pooled together.

The bottles are typically shaken for 15 seconds and sonicated for 120 s ± 5 s with a maximal power of 240 W and a frequency of 40 kHz. The liquid level in the bath needs to be above the fluid level in the sample bottles; and the number of bottles cannot exceed the performance rating of the sonicator.

For anaerobic cultivation, the wipes are processed as described above, except bottle sizes and the amount of used anaerobic PBST Buffer differs. All steps in which the sample tubes and bottles are opened are performed in anaerobic chamber.

After sonication, the extraction suspension can be filtered using vacuum pump onto Millipore filters (0,22 µm, 47 mm, white) to concentrate the samples. For each sample, a certain amount of extraction suspension is filtered per medium, and the filters placed on the plates

Incubation

Plates are generally incubated inverted at 32 ± 1°C (oligotrophic plates up to 14 days), anaerobic cultivation plates in an anaerobic jar or anaerobic chamber.

Counting

The aerobic cultivation plates are examined every day, oligotrophic plates up to 14 days. The colonies are counted and reported at time points 24 h, 48 h and 72 hours. The anaerobic plates are generally counted at time points 1 day, 3 days, and 7 days. Selection of visible colonies can be picked and pure isolates sent to databases (i.e. DSMZ – ESA strain collection) for identification.

Molecular analysis

Wipes are taken out of the freezer (- 20 °C) and thawed overnight in fridge (+ 4 °C). Thawed wipes are then placed using baked (24 h at + 250 °C) tweezers in baked glass bottles, and a certain amount of PCR-grade water is added to each bottle. The bottles are then placed in an ultrasonic bath, so that the water level in the bath is higher than in the bottles. The bottles are sonicated for 120 ± 5 seconds, and vortexed at maximum speed for 1 minute.

The liquid is concentrated to 200 µl using UV sterilised filters. The UV sterilisation is performed in clean bench by opening the filters and exposing them to UV light for 1 hour. Concentrating the liquid can be accomplished by centrifuging the samples at + 4 °C and 4000 g for 10 minutes for so many times that there was only 200 µl of the liquid left.

DNA is extracted from the remaining liquid using different methods, according to manufacturer's instructions. The amount of DNA can be measured

The V4 region of 16S rRNA gene can be amplified with so called Caporaso primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') using the following PCR program (as an example):

1. 94 °C 3 minutes.
2. 94 °C 45 seconds.
3. 50 °C 60 seconds.
4. 72 °C 90 seconds.
5. Repeat steps 2 - 4 35 times.
6. 72 °C 10 minutes.
7. 4 °C HOLD.

The fragments are then sequenced and the raw sequence data analysed to identify microbial signatures present in cleanrooms and hardware and to assess the microbial diversity. In short, the raw forward and reverse sequence reads are first joined together, then several quality control steps, including screening based on quality and length, aligning 16S rRNA database (i.e.Silva), pre-clustering, and

chimera checking, can be performed. The sequence data are clustered into operational taxonomic units (OTUs), and the OTUs classified using reference database.

Typical results from 16s rRNA gene sequencing are shown in Figure 5-58.

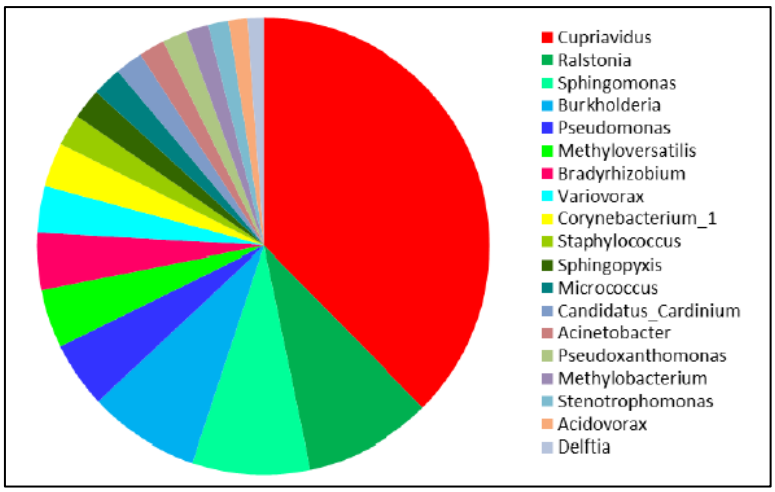


Figure 5-58: Piechart showing most abundant genera detected in dataset

5.10.4 Mitigation measures

In 2023, ESA organised a metagenomic workshop to assess the real potential of molecular biology and metagenomics methods in the field of Planetary Protection. A white paper reference ESA-TECQI-TN-2024-000152 [RD25] has been released and it can be made available upon request.

This involved reviewing the limitations and identifying knowledge gaps to be addressed before inclusion in ESA Planetary Protection procedures. Experts from different backgrounds, institutions, and organisations contributed to the workshop, including space and government agencies, industrial partners, subject matter experts, academics, and end-users.

This workshop offered an international forum to coordinate inputs for delineating a long-term strategy and framework for the update and inclusion of new techniques in the Planetary Protection domain. The workshop complemented the activity of the international scientific community, as well as international space agencies, who are also actively investigating this approach. The workshop participants recommended that ESA consider the systematic implementation of activities on metagenomic.

The need for identifying and quantifying problematic species for Planetary Protection, i.e. those capable to survive the journey to outer space, replicate and contaminate celestial bodies, and allow probabilistic risk assessment models to truly represent biodiversity, has set stringent requirements for metagenomic sequencing or any combination of methods aiming to complement the current spore assay.

In order to take full advantage of the use of molecular biology and metagenomic sequencing, the workshop experts identified areas where future work is needed for the method to be used for Planetary Protection:

1. Low biomass.
2. Intrinsic contamination of reagents.
3. Bioinformatics, including need of databases' and libraries from low biomass environments.

Low biomass

Biologically controlled cleanrooms and spacecrafts destined to visit other planets have been proved to be extremely clean, as highlighted by ESA "biodiversity knowledge" campaign performed in the past decades and using 16S rRNA gene NGS (next generation sequencing). That is due to use of HEPA (and

carbon) filtrations in cleanrooms for astrobiology missions, strict procedures used by personnel when assembling space hardware and frequent cleaning. DNA concentrations were very often below detection limits, i.e. not enough biomass was found in the cleanroom to successfully perform the molecular analysis.

Low biomass environments are seen as a major area where additional work is needed in assessing and managing problematic biological contaminants. The issue is further exacerbated by the fact that spacecrafts have even lower bioburden levels than cleanrooms' surfaces (1-2 order of magnitude less).

Different options have been suggested by workshop experts. These included:

- Sampling of extended areas (i.e. scraper system).
- Additional sampling of "dirtier" areas in cleanrooms (i.e. changing rooms, airlocks), not only the main AIT (assembly integration and testing facilities).
- Possibility of sampling non flight models (i.e. qualification, engineering, or structure thermal models), as these are generally not as clean as flight hardware.
- Sampling of ground support equipment (both electrical and mechanical).
- Sampling of gloves and personnel microbiome.
- Spike-in (i.e. with lambda phage DNA).

Inclusion of metagenomic campaigns, at different phases of spacecraft assembly (commissioning, procurement phases, main AIT, environmental test, and final launch campaign) was suggested to be added in addition to current biological independent verification campaigns. That can support the creation of a high-quality metagenome- database from PP-relevant (low biomass) environments. The existence of a genome databases and libraries can be the basis of reliable phenotype predictions. These predictions can then better refined, once more data become available and models are trained.

Planetary Protection relevant microorganisms are those alive and capable of reproduction, i.e. viable. Consensus was reached by workshop experts that viability testing can be performed with samples treated with PMA (propidium monoazide) before DNA is extracted. PMA has been successfully applied in combination with PCR to discriminate cells with intact and damaged membranes in spacecraft assembly facilities [RD26], [RD27], [RD28] and [RD29]. For halophilic bacteria (already isolated in cleanrooms) PMA cannot work to discriminate dead from live cells, as NaCl inhibits PMA from inhibiting PCR amplification [RD30].

Reagent contamination

Reagent contamination as a challenge for metagenomics analysis in low biomass environments. The obvious solution identified can be a sound control during the process, procurement of ultrapure reagents. In particular, if multiple displacement amplification is used due to low biomass, then the amplification phase is especially vulnerable. While this is seen as essential for low biomass environment, i.e. cleanrooms, it is still an additional step where contamination of DNA (and biases) can be introduced. Furthermore, with amplification phases, reliable quantification of problematic species cannot be possible.

Bioinformatics

Despite the notable advances in metagenomics methods over the last decade, smaller attention has been paid to the standardization of bioinformatics methods. Given the difficulties to find consensus on a specific tool and the many variables involved in the process, workshop participants agreed on providing flexibility to the election of appropriate software tools (also considering the fast market changes). Full consensus was reached though regarding the need of a transparent workflow tool, where all the relevant steps and critical parameters are identified and recorded. A standardized (metagenomic) pipeline,

where scientist only modifies a defined set of parameters that is to be reported at the end of the analysis can be an option, as can also result in an increased automatization of the data analysis process.

The ESA funded strain collection at DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) has been identified as an important starting point to expand current capabilities. A genome catalogue of these isolates has been considered by workshop experts strategically important for Europe; and the recommendation is to extend the scope of existing strain collection (i.e. DSMZ) to adapt for metagenomic purposes and built up a corresponding data base including phenotype predictions for these strains. Future activities can be focused on sequencing all these isolates with metagenomics techniques.

Toward alternative methods for future missions

There is not a single universal method able to tackle all Planetary Protection and mission constraint needs at the same time (i.e. rapid responses, phylogenetic and functional information, cost effectiveness, etc.). Workshop experts converged on the need to develop an internationally agreed hybrid approach, where a combination of metagenomics and rapid quantitative methods are used together. That way limitations of single methods can be minimized.

In fact, while there are specific methodologies to determine quantities with whole genome sequencing, these generally take long time. Two methods were discussed and deemed capable for reliable quantification:

Quantitative PCR (qPCR), as it can provide fast result and it is relatively cheap. The drawbacks are related to primer bias, varying rRNA gene copy numbers (single copy genes) and conversion to cell numbers.

Digital droplet PCR (ddPCR) provides absolute quantification. However, primer bias is still an issue. Despite this limitation, it remains though a very sensitive method which allows detection of rare contamination events. Also, it is commonly used to quantify the number of copies of a gene or mutations and notably it eliminates the requirements for standard curves. ddPCR gives absolute copy numbers per cell; and the droplet partitioning reduces bias from amplification efficiency and PCR inhibitors. The problem of conversion to cell numbers remains.

The whole process needs to be standardized, agreed and verified.

Additional workshops on the topic have been held with ESA's international partners, ("Metagenomics in Spaceflight: establishing an implementation roadmap", 2025) and are planned in the future, with the aim to advance the use of molecular biology in planetary protection practices.

5.11 Organic material inventory

5.11.1 Examples of organic material inventory requirements

Typical organic material inventory requirements for Planetary Protection can be found in ECSS-U-ST-20C clauses 5.3 and Annex H.

Clause 5.3.1 Moon missions

a. *An organic materials inventory of bulk constituents present in quantities above the limit agreed with the PPAA shall be provided by the project in conformance with DRD in Annex H.*

NOTE This also applies to missions not going to the Moon but having a final disposition that would end up on the Moon.

Annex H. Clause H.2.1b

b. *For missions to the Moon, including fly-by-gravity assist, a description of the products released by the propulsion and life-support system, as applicable, into the Lunar environment shall be provided, including:*

1. *A quantitative and qualitative description of the major chemical species, and*
2. *An indication of the minor chemical species and quantity.*

Clause 5.3.2 Mars missions.

Clause 5.3.2.1 General requirements

a. *An organic materials inventory of bulk constituents present on the spacecraft in quantities ≥1 kg shall be provided by the supplier in conformance with DRD in Annex H.*

NOTE This requirement is not applicable in case requirement 5.3.2.1e.1 is met.

b. *A 50-gram sample for each organic material used on the spacecraft in quantities ≥25 kg shall be provided by the supplier and stored by the customer under controlled conditions for 50 years after launch.*

NOTE This requirement is not applicable in case requirement 5.3.2.1e.1 is met.

Annex H. Clause H.2.1

a. *The organic material inventory shall include the following for each organic material present above a specified limit agreed with PPAA:*

1. *Identity;*
2. *Chemical composition;*
3. *Usage, with respect to product tree;*
4. *Mass estimate using the mass codes specified in ECSS-Q-ST-70-01;*
5. *Rating and reference for outgassing for each item using ECSS-Q-ST-70-01;*
6. *Supplier for each item.*

5.11.2 Rationale for the requirement

The search for life in outer space is associated with the detection of organic molecules. These, by definition, are those containing carbon compounds, the building blocks of biological systems. Planetary Protection is historically linked to bioburden control; a considerable amount of investments is allocated to understand biological contaminants sources and methods to reduce their level below acceptable limits. However, organic contamination control is (arguably) equally important to avoid false positives (i.e. contamination is mistaken for true life) and false negatives (terrestrial contaminants create a high background on the instrument, masking a low indigenous sign of extraterrestrial life).

All microbial contamination is organic, however, the contrary is not true. As an example, spacecrafts, landers and rovers are built using large quantities of adhesives, pottants, coatings, polymers, etc. that outgas under vacuum and can cross contaminate cleanliness-critical areas. Despite these organics are "abiotic", i.e. coming from non-biological sources, hence not able to replicate, they are still capable to contaminate ultra clean zones, and affecting GC-MS (Gas Chromatography Mass Spectroscopy) measurements.

The search for organic molecules is therefore essential to understand the chemical evolution and origin of life in our Solar system. Requirements are therefore needed to limit, identify and curate organic contaminants in space missions. It is important to note that maintaining archives of organic materials used in space missions is as important as actively limiting their amount (i.e. contamination control). Scientists can, in fact, design experiments, produce background data or replicate analysis on ground, that can be extremely helpful once missions' results become available.

Existing contamination control approaches have historically been developed mainly for Mars. At the dawn of space exploration, cleanliness and contamination scrutiny was introduced to increase spacecraft reliability and minimise the risk of mission loss, for e.g. due to malfunctioning of valves in propulsion systems, electronics, mechanical actuators. Evolution of mission needs led to cleanliness and contamination (organics) control requirements placed to avoid deterioration in performances of optics and lenses, or high frequency lasers used for weather forecast satellites (ESA Aeolus). For the latter, an incorrect selection of organics can lead to the so-called "laser induced contamination" or LIC, resulting on creation of unwanted organics and loss of laser functions.

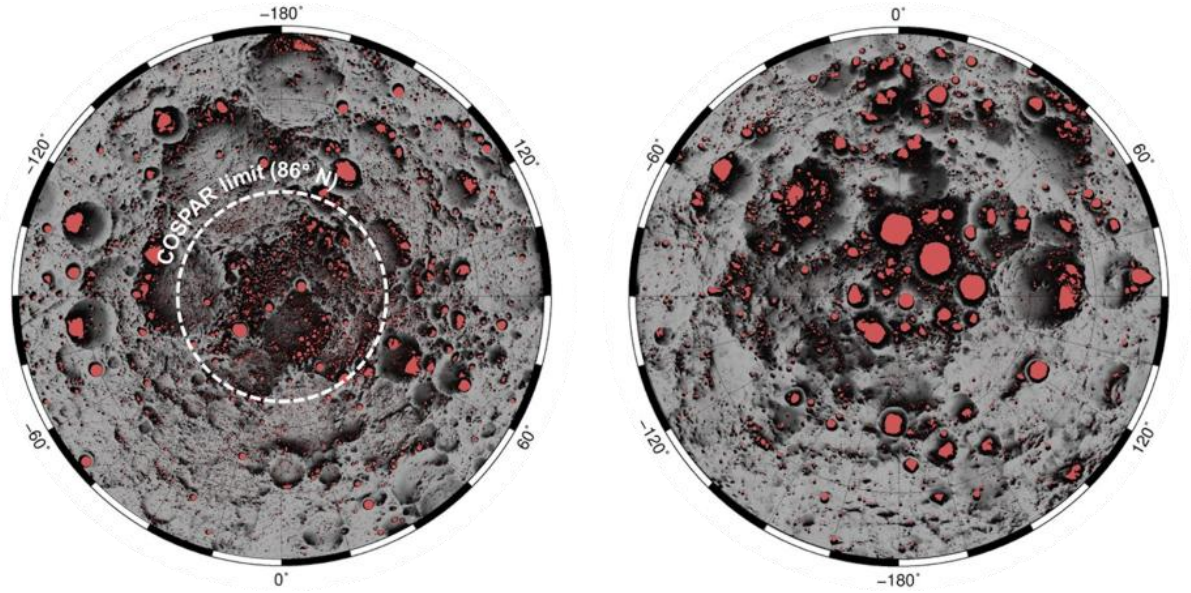
It is important to highlight that the requirements discussed in this Section 5.11.2 are not related to preserve optics integrity. Control of organics in Planetary Protection is linked to a different goal compared to cleanliness and contamination. That is to identify (via cataloguing) the introduction of terrestrial contamination that can affect the analysis of Mars samples. On ExoMars Rosalind Franklin Rover for example, tests and analysis of the continuous organics' accumulation inside the UCZ volume are being performed using a fluidic ground support equipment and GC-MS technology. The resulting values are then compared to the TOC – Total Organic Carbon thresholds set for not exceeding the instrument sensitivity.

Organic contamination knowledge is not related to Mars mission only. The way an organic catalogue is used vary significantly depending on target bodies. For icy worlds (i.e. Jupiter and Saturn Moons) major uncertainties remain. The potential for surface or subsurface transport of chemical contaminants remains a critical knowledge gap. While organic compounds do not replicate and can eventually be diluted below detection thresholds over time, their persistence and mobility still represent a source of analytical interference for future scientific investigations. Also, the high radiation environment can likely change their chemistry.

For the Earth's Moon, scientific interest is mainly focussed on volatiles, likely to be trapped on ices in Permanent Shadowed Regions (PSRs) or poles. They can be a record of pre-biotic organic materials delivered to the Earth-Moon system. Lunar polar ices irradiated by cosmic rays provide a natural laboratory for the synthesis of organic molecules (i.e. a natural Miller-Urey like experiment). The Moon is therefore of great astrobiological interest, and Lunar poles are the most

easily accessible locations in the Solar system. It is important to preserve Lunar poles from disturbance of terrestrial volatiles, coming from rocket propellants or spacecraft materials. The northern latitude limit for COSPAR PPP Category II(b), 86 °N, is indicated; the corresponding southern limit, 79 °S, lies outside the area shown. Data from https://pgda.gsfc.nasa.gov/products/69 (Mazarico et al.).

Figure 5-59 shows some specific locations for PSRs mentioned in the COSPAR PP policy.



The northern latitude limit for COSPAR PPP Category II(b), 86 °N, is indicated; the corresponding southern limit, 79 °S, lies outside the area shown. Data from https://pgda.gsfc.nasa.gov/products/69 (Mazarico et al.).

Figure 5-59: Locations of permanent shadow (marked in red) overlaid on average Solar illumination (grayscale) within 10° latitude of the Lunar North Pole (left) and South Pole (right).

In addition to organic inventories, ESA Planetary Protection have developed computer simulations to predict how gases released by a spacecraft spread across the Moon. The main objective of these studies is to assess the potential impact of organic contamination from Lunar landers on scientific research of Lunar ice chemistry on polar craters. Preliminary simulation results, based on ESA Argonauts missions, suggested that these gases can travel across the whole Moon by "hopping" through its surface, and can end up in these craters within a relatively short period. Remarkably, even if the landing of Argonauts occurs at the South Pole, a significant amount of contaminants can still reach the opposite pole (north). These insights contribute to the development of effective strategies to protect the Moon's unique environment, ensuring the success of future scientific discoveries.

More information related to this work is presented in Annex A.1. Despite the absence of specific requirements enforced on mission teams for modelling organic transport mechanisms for Moon's missions, information is still presented within this handbook, in case Lunar mission teams are interested in use that model on a voluntary basis.

5.11.3 Methods to assess compliance

Mission teams can use the templates in Annex B to provide information about the quantity and type of organics for different missions as needed (Moon, Icy Worlds, Mars).

For Mars mission, for quantities over a certain threshold (50 gr), a physical inventory is also needed. For that, prime contractors are instructed to deliver such organic quantities to customers (i.e. ESA).

5.11.4 Mitigation measures

It is important to note that declared material (DML), Mechanical (DMPL) and Processes (DPL) lists are produced and updated as part of key project reviews. These remain the main source for extracting information about organics, their mass and volumes (to estimate encapsulated bioburden). `

One of the issues that can be encountered when complying with such requirements is duplication and inconsistency of information, due to presence of different documents, i.e. DML/DMPL/DPL and organic inventories. To avoid that, mission team can extract information directly from materials and processes lists, and establishing a clear documentation hierarchy where, in case of different information between documents, the DML/DPL takes precedence. This strategy can avoid the overlooking of organics, for e.g. typically those added during Assembly Integration and Testing (AIT). In fact, these are not necessarily declared or even known at design level, as more information becomes available only at later mission stages. Material and processes control boards in place as part of normal business activities in projects, can make sure that organics used in AIT are also included in the deliverables, so that a complete, "as built" organic inventory is provided.

For materials that need to be physically delivered to customers (for quantities >50gr), there are some guidelines that can be considered:

1. It is preferred to deliver the samples along with flight hardware.
2. UHF (Ultra High Vacuum) Aluminium foil can be used to individually seal the samples.
3. Folding aluminium foil twice is recommended to minimise oxygen exposure. The aluminium foil packaged samples can then be individually sealed into ultra-low outgassing polyethylene bags (x2) to reduce environmental exposure. Labelling of individual packaging can be performed on the first layer of ultra-low outgassing polyethylene bag, so that labelling is preserved by the outer polyethylene bag.
4. Packaging and storage of sample materials can be performed using the same environmental conditions as those used during the built and storage of flight hardware (i.e. ISO 8 or better).
5. Sample materials can be mixed, prepared and cured in the same way as the materials used on flight hardware, and using roughly the same cross-section and configuration as used on flight hardware.
6. It is important to label the samples, including as a minimum information about mixing, preparing and curing conditions, bake-out – if any, configuration, etc.
7. Separating the 50 gr samples into multiple fraction can be requested by the PPO to minimise the chances of the entire sample being compromised by mishandling or wrong storage conditions used (example: 5 ten-gram samples each).
8. In case of paints, providing material samples of the paint coated on clean aluminium foil is the preferred option. For liquid samples, clean glass vials with PTFE cap liners or clean crimp top vials with PTFE sealing surface can be used. For solid samples, fold up into clean aluminium foil, assuring the edges are folded at least twice. For sample materials sealed in glass ampules (i.e. derivatising agents) leaving it sealed in the original ampule is the preferred option to minimise the chances of cross contamination.
9. Exemplary blank packages of aluminium foil, packaged as the sample materials can be requested by the PPO on a case-by-case basis to assess the contamination of the aluminium foil.

5.12 Restricted Earth return: potential unsterilised contaminants release

5.12.1 Examples of restricted Earth Return mission requirements

Typical restricted Earth Return mission requirements for Planetary Protection can be found in ECSS-U-ST-20C clauses 5.3.

5.3.2.3 Mars sample return missions

a. Requirements in clause 5.3.2.2.3 shall be applied to the outbound leg of a Mars sample return mission.

b. The severity of potential consequences of releasing unsterilized material from Mars and flight hardware that has been exposed to unsterilized material from Mars into the terrestrial environment shall be categorised as catastrophic in accordance with ECSS-Q-ST-40.

NOTE The categorization of this severity level is not because a catastrophic consequence is expected but only following the precautionary principle due to the large number of unknowns.

c. Safety critical functions as defined in 3.2.19 shall be treated as severity function criticality level 1 in accordance with ECSS-Q-ST-40.

d. The probability that a single unsterilized martian particle of ≥ 0,01 μm in diameter is released into the terrestrial environment shall be ≤ 1x10-6 for the first 100 years after launch from Mars.

NOTE 1 Rational for this requirement is to "break the chain of contact" between Mars and Earth.

NOTE 2 Source and context for numerical values in [5].

NOTE 3 The term 'particle' includes material from Mars and flight hardware exposed to material from Mars.

NOTE 4 If the size requirement of 0,01 μm cannot be met without decreasing the overall level of assurance for the non-release of such a particle, the release of a single unsterilised particle of up to 0,05 μm can be considered as a potentially tolerable systems-level adjustment, assuming that it has been demonstrated that this size is the lowest achievable at a reasonable cost (BAT approach) and it has been independently reviewed.

e. *A quantitative PRA shall be conducted as part of evaluating the risk of releasing unsterilized material from Mars and flight hardware that has been exposed to unsterilized material from Mars into the terrestrial environment.*

f. No unsterilised portion of the materials returned from Mars shall be released from containment unless approved by the PPAA.

5.3.3.2 Europa and Enceladus sample return missions

b. The severity of potential consequences of releasing unsterilized material from Europa or Enceladus and flight hardware that were exposed to unsterilized material from Europa or Enceladus into the terrestrial environment shall be categorised as catastrophic in accordance with ECSS-Q-ST-40.

NOTE The categorization of this severity level is not because a catastrophic consequence is expected but only following the precautionary principle due to the large number of unknowns.

c. Safety critical functions as defined in 3.2.19 shall be treated as severity function criticality level 1 in accordance with ECSS-Q-ST-40.

5.3.4 Missions to small Solar system bodies

5.3.4.1 General

b. *The mission shall be categorized as "restricted Earth return" if the answer to all the following six questions is "no" or "uncertain", otherwise the mission is categorized as "unrestricted":*

1. *Does scientific evidence indicate that there was never liquid water in or on the target body?*
2. *Does scientific evidence indicate that metabolically useful energy sources were never present?*
3. *Does 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 scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures >160 °C?*
5. *Does scientific evidence indicate that there is or was sufficient radiation for sterilization of terrestrial life forms?*
6. *Does scientific evidence indicate that there has been a natural influx to Earth, via meteorites, of material equivalent to a sample returned from the target body?*

5.3.4.2 Small solar system bodies restricted Earth return missions

5.3.4.2.2 Requirements

b. *The severity of potential consequences of releasing unsterilized material from specific small Solar system bodies and flight hardware that has been exposed to unsterilized material from specific small Solar system bodies into the terrestrial environment shall be categorised as catastrophic in accordance with ECSS-Q-ST-40.*

NOTE The categorization of this severity level is not because a catastrophic consequence is expected but only following the precautionary principle due to the large number of unknowns.

c. *Safety critical functions as defined in 3.2.19 shall be treated as severity function criticality level 1 in accordance with ECSS-Q-ST-40.*

d. *No unsterilised portion of the materials returned from Mars shall be released from containment unless approved by the PPAA.*

5.12.2 Rationale for the requirement

Requirements discussed here are specifically for Mars mission. However, the main objective of these constrains, i.e. protecting Earth, are the same for any return mission from astrobiology targets with the potential of bringing harmful contamination back to our biosphere. As such, the probabilistic risk threshold of "1 in a million" is expected to be the same for any other Earth-restricted sample return mission.

A Mars Sample Return (MSR) campaign is a response to the long-running scientific objective to better understand Mars. By collecting and returning to Earth a rigorously documented set of Mars samples, scientists can have access to the full breadth and depth of analytical scientific instruments available in terrestrial laboratories. The investigations are free of the mass, volume, and power constraints that limit in-situ instruments. The resulting investigations of these returned samples can enable breakthrough advances in:

- The search for ancient and/or extant life on Mars.
- Understanding the origin and evolution of Mars as a geological system.
- Understanding the processes and history of climate on Mars, and
- Preparing for human exploration of Mars.

Samples can be selected based on their geologic diversity, astrobiological relevance, and biosignature preservation potential, and detailed in situ observations made at the time of sampling can establish the field context for each sample.

An MSR architecture typically involves different elements, launch campaigns. These can be:

- A caching horizontal or vertical mobility mission (i.e. rover, helicopters).
- A sample retrieval mission, consisting of a lander.
- A Mars ascent vehicle launched from Mars surface.
- An orbiter bringing back the samples to Earth.

The potential release of unsterilised materials from flight hardware that has been exposed to Mars needs safety considerations be an essential part of the design and operations of Mars Sample Return missions.

Key requirements placed by ESA to limit the risk for Earth's environment and public health, are based on particle size (higher or equal to 10 nm) and a probability of that particle being released unsterilised of 1 in a million. An independent study requested by ESA to the European Science Foundation (ESF) and The European Space Sciences Committee (ESSC), was commissioned in 2012 and updated in 2025. The mandate of that study group was to recommend the level of assurance for the exclusion of an unintended release of a potential Mars life form into the Earth's biosphere for MSR missions.

Following an NRC-SSB report in 1999 "Size Limits of Very Small Microorganisms: Proceedings of a Workshop" that established that the value for the maximum particle size was 0,25 ± 0,05 μm, this value was revisited and re-evaluated by ESF-ESSC based on new discoveries made in past decades in microbiology, finding microbes of concern in the range of 0,10 – 0,15 μm. The ESF-ESSC study also recommended that containment of particles larger than a given size was the most appropriate constraint to be considered when designing a Mars Sample Return missions.

Dimension of microbial cells is typically expressed as the diameter or volume of coccoid cells, or length, diameter and volume of rod-shaped cells. Virus particles are small infectious agents that can replicate only inside living cells. Similar to cells, virus dimensions are also measured as capsid size or length for head-tail bacteriophages and rod-shaped and filamentous viruses (and genome size).

A review of the microbial size range was done in 2012 and repeated in 2025. The latter study confirmed that the fundamental size thresholds established in 2012 can be maintained. A minimum particle size of

0,01 µm (or 10 nm) corresponding to a maximum allowable release probability of 10^{-6}, and 0,05 µm (or 50 nm) as the zero-release threshold can be applied (as shown in Figure 5-60)

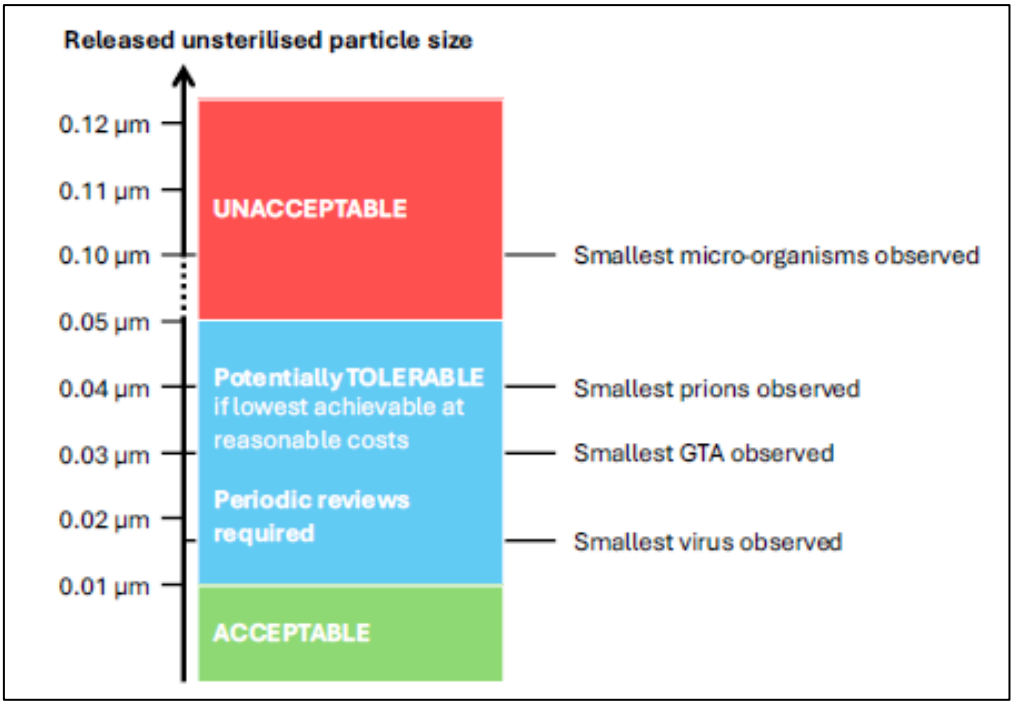


Figure 5-60: Extract from the ESA ESF 2025 study. Representation of acceptable, tolerable and unacceptable size range for unsterilised particles released for Planetary Protection purposes.

With regards to the probability of releasing such particle sizes unsterilised, the precautionary principle is applied at ESA for Mars Sample Return. That is defined by the World Health Organization (WHO) as "a risk management policy applied in circumstances with a high degree of scientific uncertainty, reflecting the need to take action for a potentially serious risk without awaiting the results of scientific research". When deciding what it can be considered an acceptable tolerable level of risk, both independent ESF studies agreed that "one in a million has been accepted and considered by regulators as the gold standard to demonstrate excellence in risk management.

Figure 5-61 below shows where the "one-in-a-million" requirement is used in different regulatory frameworks. It is important to highlight that there is general consensus in the international scientific community that catastrophic consequences to Earth are not expected if one or more particles larger than 10 nm be released to our biosphere. ESA are only following the precautionary principle due to the large number of unknowns.

Organisation	Scope	Risk	Limit of acceptability
US Environmental Protection Agency (EPA)	'Superfund' programme to clean up uncontrolled hazardous waste sites	Lifetime cancer risk to an individual exposed to a cleaned-up site	10^{-4} to 10^{-6}
US Food and Drug Administration (FDA)	Food produced from animals exposed to carcinogenic compounds	Lifetime cancer risk to an individual eating food from animal exposed to carcinogenic compounds	10^{-6}
European Chemical Agency (ECHA)	Exposure to chemicals	Lifetime cancer risk levels to an individual exposed to chemicals	10^{-5} to 10^{-6}
EU Air Quality Directive	Exposure to carcinogenic compounds	Lifetime cancer risk levels to an individual exposed to carcinogenic compounds	10^{-6}
EU Drinking Water Directive	Exposure to carcinogenic compounds	Lifetime cancer risk levels to an individual exposed to carcinogenic compounds	10^{-6}
World Health Organisation (WHO)	Drinking Water Quality	Annual individual DALY (disability-adjusted life year) – provides a way to approach overall disease burden on people by compiling various effects (degree of illness to death) resulting from a single source	10^{-6}
UK Health and Safety Executive (HSE)	General guidance for setting a limit between unacceptable and tolerable risk	Annual individual risk of dying	10^{-6}
Australia Environment Protection Authority	Offsite Individual Risk from Hazardous Industrial Plant	Annual individual risk of dying as a result of an industrial accident	10^{-6}
International Standards Organisation (ISO)	Reliability for structures (buildings, bridges...)	Annual individual risk of dying as a result of the collapse of a structure	10^{-6}

Figure 5-61: One in a million requirement used in different regulatory frameworks (extract from ESF-ESCC study 2012)

The one-in-a-million risk threshold is therefore considered a practical convention. However, it is not excluded that future assessments can be performed by ESA to acknowledge societal perceptions of acceptable risk, as this can shift or change in view of recent global pandemics and increasing systemic fragility of the biosphere. In that respect, targeted studies can be performed to understand better public perception, by comparing past assessments and examining how institutional trust influences public support for Planetary Protection measures (as recommended in the ESA – ESF 2025 study).

As it stands today, one in a million is aligned with international standards for biosafety and risk management, as it is designed to account for multiple layers of failure probability across spacecraft systems, containment units, and Earth entry processes.

5.12.3 Methods to assess compliance

The measures to reach compliance against the so-called "backward contamination" requirements discussed in this Section 5.12.3 are specifically related to the ESA ERO – Earth Return Orbiter, in the frame of the former ESA & NASA MSR architecture presented in Figure 5-62.

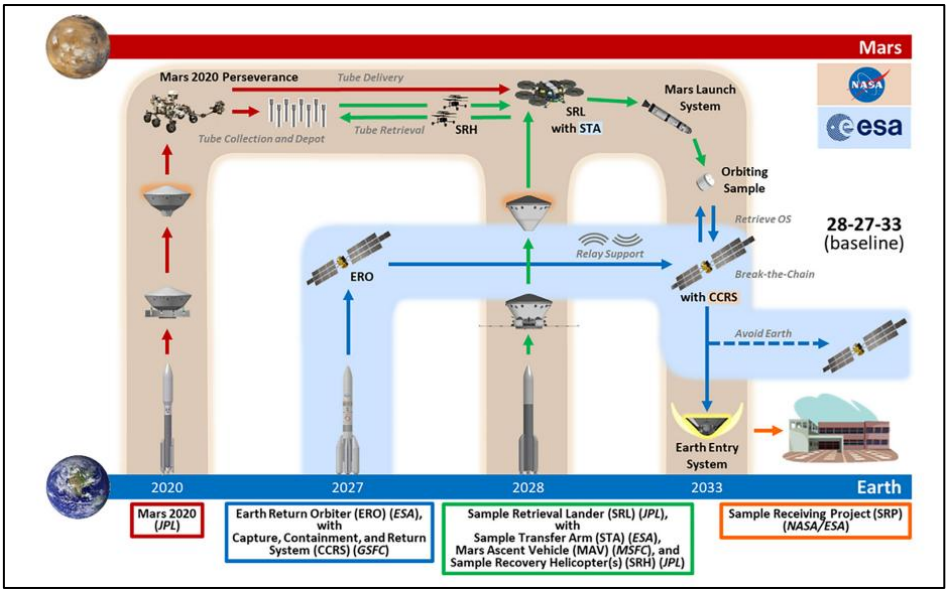


Figure 5-62: Element of the (former) planned ESA NASA MSR campaign. In blue, the ESA ERO element.

The Earth Return Orbiter (ERO) mission was planning to use Solar-electric propulsion for a two-year transfer to Mars. Upon arrival, the Orbit Insertion Module (OIM) was going to perform a chemical Mars orbit-insertion into highly elliptical orbit, after which the OIM is jettisoned. Subsequently, ERO was going to spiral down to a circular orbit to provide telecommunications support for Mars Sample Return (MSR) surface operations, track the Mars Launch System, and detect and rendezvous with the Orbiting Sample (OS).

Using its rendezvous sensors (LiDARs and cameras) and radio-beacon support, ERO have captured the OS with the Capture, Containment, and Return System (CCRS). After transferring the sample into the Earth Entry System (EES), ERO was going to jettison CCRS elements and departed Mars using solar-electric propulsion. The return journey typically takes two years.

An overview of the ERO mission trajectories and phases is presented in Figure 5-63.

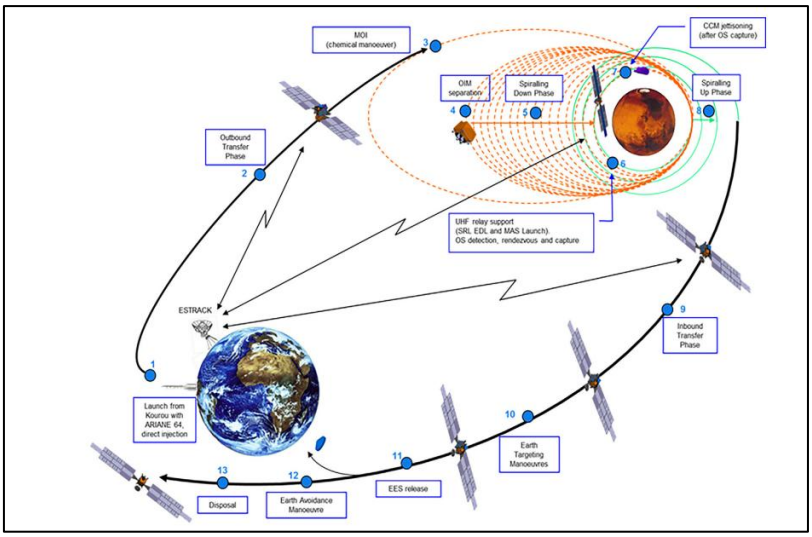


Figure 5-63: ERO mission baseline with main trajectories shown

About one week before Earth arrival, ERO was going to release the EES – Earth Entry System, then performed an Earth-avoidance manoeuvre, so that only the EES enters Earth's atmosphere. The EES was expected to land at the Utah Test and Training Range (UTTR). Afterward, ERO was going to perform a disposal manoeuvre, placing itself in a safe heliocentric orbit for at least 100 years to prevent accidental delivery of Martian material to Earth.

ERO was designed to have a modular architecture, consisting of three main components:

Return Module (RM): Hosts communications antennas, large solar arrays, electric and chemical thrusters, computers, fault-management systems, and accommodations for CCRS and the Rendezvous Sensor Suite.

Orbit Insertion Module (OIM): Provides powerful chemical propulsion for Mars orbit insertion and is later jettisoned

Rendezvous Sensor Suite (RSS): Two LiDARs and two narrow-angle cameras for detecting, tracking, and guiding the capture of the Orbiting Sample.

The Planetary Protection process used at ESA to break the chain of contact with Mars is designed to be phased, independent and aligned with major key reviews (e.g. PDR, CDR, QR).

For the ESA/NASA MSR campaign the backward Planetary Protection hazards were identified and the responsibility to prevent them was assigned and agreed between partners via a Memorandum of Understanding (MoU):

a. ESA responsibility:

   1. ERO inadvertently enters the Earth/Moon system with harmful matter

b. NASA responsibility:

   1. ERO and/or EES emit harmful matter from intentionally uncontained hardware to the Earth/Moon system.
   2. Supposedly contained harmful matter is released on an Earth-return trajectory or during Approach Entry Descent (AED) or landing of the EES.
   3. Supposedly contained harmful matter is released after the landing of the EES.

Only ERO implementation approach to prevent hazard **a)** within perimeter or ESA responsibility can be described herein.

ERO inadvertently enters the Earth/Moon system with harmful matter

The ERO project is attempting compliance with the requirement to release one or more unsterilized Martian particles with diameters ≥ 10 nanometres into Earth's biosphere with a probability of ≤ 1×10^{-6} for the first 100 years after launch from Mars. This is attempted by avoiding that any part of ERO enters the Earth biosphere with or without release of the EES (Earth Entry System).

There are two key assumptions that ERO project made:

1. Release of Martian particles onto Earth's biosphere is a hazard level 1.
2. ERO carries unsterilised particles, hence passive sterilisation due to several years in space under several stressors (e.g. radiation, UV, desiccation, etc.) is not considered.

To demonstrate compliance of the mission with such requirement, a Probabilistic Risk Assessment (PRA) has been performed, which attempts to quantify and identify all scenarios leading to the release of unsterilized Martian particles. For the PRA, an event sequence diagram was created to determine all event sequences leading to the release. The probabilities for the transitions between events in the sequence were determined by fault trees, and the events in the fault trees quantified via a reliability analysis.

It is important to note that the PRA is not attempting an actual quantification of the events, but rather it is as a tool used to determine if acceptable hardware redundancy is achieved and to guide the concept of operations (e.g. what to do if a redundant component is lost), considering historic reliability data. This is due to the difficulty to adequately determine the probability of scenarios for a new mission, with a new system and with new design. However, a strength of the design is the ability to monitor the spacecraft health and functionality during the mission, and before the decision to enter safety critical phases is made (prior to leaving the Mars orbit to return to Earth).

In addition to the PRA, a hazard analysis has been performed. This is needed to: 1. identify scenarios leading to ERO or any parts of it entering the Earth biosphere, 2. define hazard controls to prevent these scenarios and 3. define verifications of these hazard controls. Final hazard reports are needed to demonstrate compliance with the qualitative requirement that only a combination of 3 independent failures or errors can lead to the release of unsterilized Martian particles (2 fault tolerance).

Not all scenarios leading to the entry of ERO parts into Earth's biosphere include a minimum combination of 3 independent failures or errors (e.g. loss of structural integrity or collision with space debris). The approach to prevent these scenarios is either robust design and flight hardware verification (e.g. structural integrity) or verification of low probability (e.g. collision with space debris), which is also documented in the hazard reports.

The PRA, hazard analysis and hazard reports (documenting the hazard analysis outcome, the hazard controls and verifications) have been reviewed by ESA in regular project reviews and by the independent ESA Planetary Protection Re-Entry Safety Review Panel (PPRSRP) as additional project independent entity (see next Section 5.12.4 for more information about the role of the ESA PPRSP.

ERO is a complex, computer controlled cyber-physical system. To ensure the traditional hazard analysis methods used by the project (FTA, FMEA), which were developed for electromechanical systems, do not overlook scenarios leading to the release, the ESA PPRSRP acquired independent safety engineering experts to perform an additional hazard analysis using the Systems Theoretic Process Analysis (STPA) method, which was developed for exactly such cyber-physical systems.

5.12.4 Mitigation measures

The complexity of an Earth's restricted sample return mission entails high level of coordination among different stakeholders, inside and outside organisations. It includes involvement of national and international regulatory authorities, independent scrutiny and supervision of biosafety programs established to fully mitigate biological risks.

The main mitigation strategy for Earth restricted sample return mission is using the concepts of precaution and prevention. The so called "precautionary principle" states that action needs to be taken to prevent harm even if some cause-and-effect relationships are not fully established scientifically. The precautionary principle therefore seeks to shift health and environmental policy from a strategy of "reaction" to a strategy of "precaution". Of course, proving complete safety is usually impossible. Rather, most public health decision making necessarily involves a "balance of evidence" approach in which the likely risks are judged to be acceptably small, rather than zero. Thus, the precautionary principle involves anticipatory, forward-looking action rather than reactive impeding of progress. Thus, the precautionary principle, provides a useful compass to guide ESA (and public health) decisions under uncertainty.

Communication to the public is also an important aspect to be considered. Space scientists and engineers deal with risk in a different way than non-experts. For experts, judgment of risks is inherent to a statistical measure (from high to low). For lay people, a multitude of other factors play into it, including societal and cultural aspects. Fears for technologies are driven by the concern of potentially catastrophic events not properly controlled (e.g. nuclear). Transparency in the decision-making process helps to make the risk more understandable and, therefore, acceptable. The recent Covid-19 pandemic is, without doubts, influencing public perceptions of the risk associated to bring back samples from space. An overall communication strategy, which includes specific training programs, outreaching activities and dedicated sessions for public to be able to express their opinions or fears, is deemed an important aspect for any restricted Earth returned sample missions.

Defining a safety process to follow from spacecraft design to final approval prior to launch and before receiving the returned samples from space, is one of the main tasks for backward Planetary Protection purposes. In addition to the methods described in Section 5.12.2 to ensure compliance to requirements, the ESA Planetary Protection officer has established for **Category V-r** missions a Planetary Protection re-entry safety (PPRSP). Such a panel is tasked to review ad adequately mitigate biological risks associated with the campaign, by proposing the adoption of specific measures during all phases of the mission: prior to launch, prior to entering the return phase, and prior to the release samples to the Earth-Moon system.

The ESA PPRS panel is a self-standing entity with the mandate to review and assess all public and Earth environmental safety aspects and with the authority for the approval of related safety issues.

In case of non-conformances or deviations to the ESA Planetary Protection policy and requirements, the findings of the PPRSR panel are reported to the ESA Planetary Protection re-entry safety review board (PP-RSRB) for decisions. The outcome of the Planetary Protection re-entry safety review is used as input for certification at launch and decision to release any Earth entry systems.

For ERO - Mars Sample Return mission, a review was held twice: the first one in preparation for the system level ERO CDR (2023) and the second one in 2025, following the second Independent Review Board (IRB-2) and MSR IRB Response Team (MIRT) that led to a re-architecture process during 2024.

A PPRS review is typically based on two main pillars:

1. Independent review and approval of compliance of the mission with associated PP requirements. In the case of ERO MSR, that means, an independent review of the probabilistic requirements of ERO to inadvertently release particles larger than 10 nm to the Earth-Moon system with a probability of one in a million for 100 years

2. Judge if the risk of a return campaign for public health and Earth's biosphere is acceptable, document the findings in a report for ESA to make a risk informed decision about its participation in the campaign.

The notional hierarchy of the Planetary Protection review process is shown in Figure 5-64.

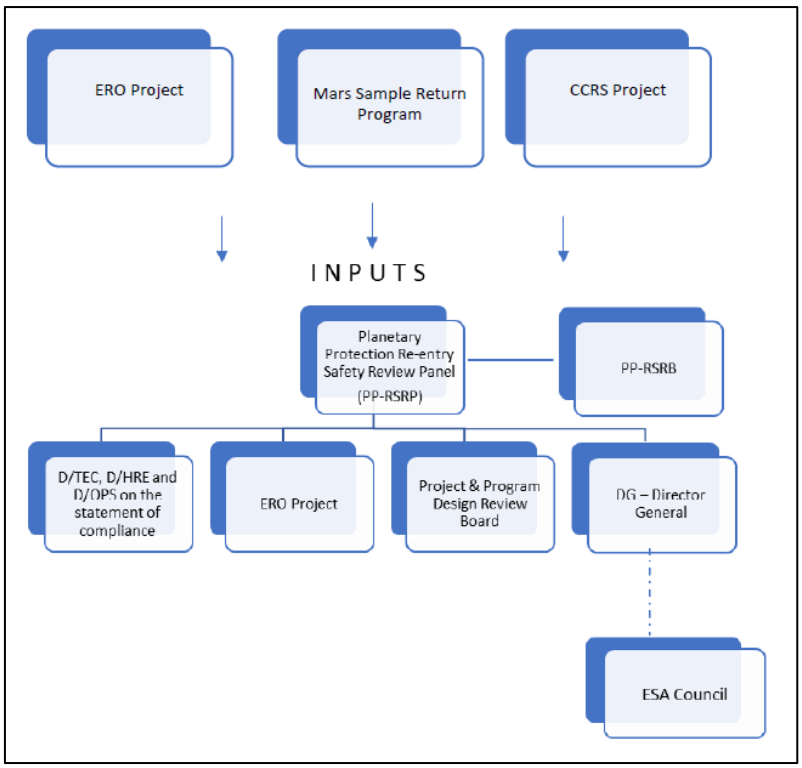


Figure 5-64: Planetary Protection re-entry safety panel – Hierarchy for ERO MSR

Duties and responsibilities of the ESA PP Re-entry Safety Review Panel are to:

- Perform, in the timeframe of the campaign, the safety review of flight elements configuration, all planned operations relevant up to the release of any Earth Entry system containing extraterrestrial samples, including model assumptions, risk assessments (i.e. probabilistic), tracking and monitoring support and the plan of notifications to relevant national and international bodies.
- Provide a notification and consultation plan to relevant national and international bodies.
- Provide an assessment and recommendation regarding the biological risk to public health and Earth's environment for the different stages of the mission: prior to launch, prior to entering the return phase of the mission, prior to authorise the release of the sample to the Earth-Moon system.
- Review the robustness and credibility of the "break the chain" concept, considering the overall "end-to-end" process.
- Verify the completeness and adequacy of risk mitigation measures.
- Verify that a clear chain of command and processes have been established for critical joint operations.
- Assess and provide recommendations to the ESA re-entry Safety Review Board on the approval or rejection of non-compliances to the ESA Planetary Protection requirements submitted by project against flight systems.
- Perform Planetary Protection requirements gap analysis versus ESA PP Policy .

- Prepare and release the final PP-RSRP report ***prior to each review/milestone*** in the program for all interested stakeholders (up to ESA DG (director general, ESA Council).

NOTE ESA Member States as stakeholders are informed at various steps of the process

The PP-RSRB (Board) is only created in case of non-conformances or deviations by the Project to the ESA Planetary Protection policy, procedures and requirements.

Detailed duties and responsibilities of the PP-RSRB, after having received a copy of the Panel Reports highlighting the Planetary Protection deviations, are to:

- Analyse the findings and confirm the panel recommendations; or provide alternative instructions, for any open issue raised to the Board for resolution.
- Establish the final conclusions of the Planetary Protection re-entry safety review in its board report.

The PP-RSRP members include ESA and external experts in spacecraft/vehicle system reliability, re-entry operations, risk assessment and management, biohazard, public health, microbiology and ad-hoc specialists nominated in consideration of mission peculiarities.

In addition, the panel membership can include as well technical representatives of the ESA directorate responsible for mission operation.

Detailed member names, their function within ESA, national space agencies or international scientific community are typically detailed in separate ad-hoc procedures.

It is good practice to hold such a review in conjunction with major project milestone (PDR, CDR, QR, etc.), but at least prior to launch, prior to entering the return phase of the program, prior to Earth entry, and again prior to authorise the release of the Earth entry system containing extraterrestrial samples.

It is important that an “end-to-end” assessment of the risks for public health and Earth’s biosphere is performed by this review, rather than covering only specific element of the mission. For that, mission partners coordination and collaboration are paramount.

Annex A
Planetary Protection implementation tools

A.1 Organic transport model for the Earth's Moon

This Annex summarises a model that can be used by project teams as a basis to predict organic molecules trajectory based on ESA Argonaut lander fuelled by a bipropellant mixture of MMH and MON - 3. Full information is contained in [RD 32].

Please see below the relevant extracts from the paper and an illustrative schematic in Figure A-1.

The model is based on the one proposed by [RD 33] and considered the last 601,3 seconds of descent of a simulated landing provided by the Argonaut team [RD 34]. Based on a Chemical Equilibrium with Applications (CEA) analysis of the Argonaut Lunar lander engine under equilibrium conditions, CH_4 is identified as the most abundant organic compound present in the exhaust. The simulation begins when the thrusters are oriented 45° relative to the axis normal to the surface, and thereafter, the exhaust gases are channelled toward the Lunar surface. The modelled descent starts at an initial altitude of 30 km and ends at a final altitude of 0 km, covering a total trajectory length of ~ 494 km. The landing is assumed to occur in 2031, in the Lunar South Pole (90° S, 0° E). The spacecraft's exhaust gas emission is simulated by generating, at every time step, $\Delta t = 5 \times 10^{-2}$ s, a new molecule, representing~ 8 × 1020 real molecules, within a spherical source region with 40 metres radius cantered at the nozzle exit.

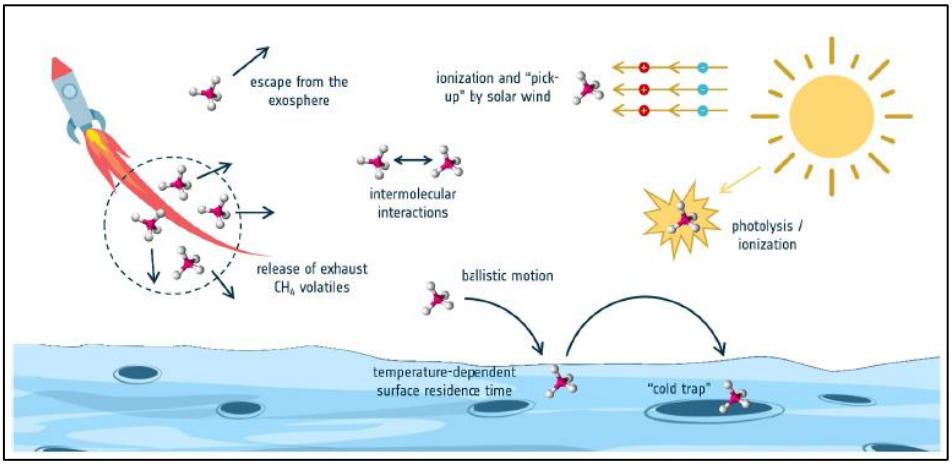


Figure A-1: Schematics of the physical processes and pathways used to model CH_4 molecules (extract from Sinibaldi and Paiva, 2025 [RD 32])

A molecule is considered to have escaped from Lunar gravity when it reaches an altitude of 10300 km above the Lunar surface, and it is thereafter removed from the simulation [33]. Molecules which cannot escape can still be dissociated or permanently lost due to photolysis or sweeping by Solar wind's electric and magnetic fields.

Simulations showed that, after an initial phase of intermolecular interaction, propagation of volatiles is based mainly on their interaction with the surface (activation energies of desorption, local surface temperatures and distribution of cold traps). 42,4 % and 11,9 % of contaminant molecules got trapped in PSRs, respectively, at the South and North poles, within 7 Lunar days (or 196 terrestrial days), while only 39,7 % and 1,5 % ended up within the corresponding latitude limits for **Category IIb** defined in the

COSPAR Planetary Protection Policy. spacecraft exhaust volatiles have the potential to distribute globally across the Moon, including the PSRs.

A.2 Probability of contamination

A.2.1 Introduction

The European Space Agency (ESA) ECSS and Committee on Space Research (COSPAR) Planetary Protection requirements for **Category III & IV** missions to Icy Worlds require a probability assessment of inadvertent contamination from viable organisms carried by spacecraft to subsurface liquid oceans of 1×10^{-4}.

Below is an example for a **Category III** mission project on how to achieve the objective of developing a suitable model for Planetary Protection risk-informed decisions.

To calculate the probability of contaminating another world,we need to break the problem down into four distinct parts. Each part needs different mathematical models and statistical techniques to provide the relevant information.

The key elements of the analysis are:

b. What types of microbes exists on the spacecraft and what is the probabilities for the numbers of each type?

c. For the numbers of each type what is the probability that they survive the journey to their target planet or moon?.

d. What is the probability of an unplanned descent to the surface of a planet and any microbes still be in a viable state?

e. If they do reach the surface in a viable state, what is the probability of them reaching liquid water and reproducing within 1000 years of their launch date?.

Microbe Classification

The number of distinct microbes that exists is large and it is impractical to identify them all and to assess their properties. One way forward is to classify microbes into groups then we define the properties for each group. Because there cannot be a single classification scheme that can work for all possible questions. Expectation is to redefine the classification scheme for each question we need to answer. We highlight how such a classification can work.

Microbes have many traits, some of which are known or tested for. Some of these traits have relevance to determining if they can survive space travel and whether they are viable on another planet. Examples of possible traits of interest include tolerance to oxygen, or elevated temperatures or reduced humidity. Some of the traits can be defined with simple discrete options, others depend on the behaviour of a microbe across a spectrum of possible values, such as the maximum constant temperature at which a microbe can survive or divide. The average differences in the values of the traits between members of a group within the classification scheme can be smaller than the average differences between members of different groups. We can therefore use the average value of a trait within a group to be representative of the whole group. It is quite possible that there will be no individual microbe that can be completely representative of the group. Meaning that no microbe has the average value for all the traits.

The traits that can be used needs discussion by the relevant scientists. The following list is given to illustrate a possible set of traits:

a. Minimum growth temperature on any medium.

b. Maximum growth temperature on any medium.

c. Maximum survival temperature on any medium in any state.

d. Minimum active water fraction that allows growth.

e. Maximum number of Freeze-thaw cycles that can be survived.

f. Maximum number of days at – 80 °C that can be survived.

g. Maximum number of days of desiccation that can be survived.

h. Maximum UV-C (J/m^2) in any media that can be survived.

i. Maximum polychromatic UV in any media that can be survived.

j. Maximum x-ray in any media (Gy) that can be survived.

Microbes can be classified into groups that are defined by their properties. The classification scheme can be redefined for each question we need to answer.

These traits define a multidimensional mathematical space where individual microbes occupy a single point in the space. It is unlikely that microbes are uniformly spread across the space. There are probably parts of the mathematical space that are devoid of microbes and other parts that will see them clustered close together. We need to subdivide the space of traits up in such a way that we form groups of similar trait values. There is no unique method for doing this, but below we provide a simple approach.

Figure A-2 displays an example of what we can have with just two possible traits and 15 microbes. Figure A-3 shows how this 2D space can be divided into 12 categories. Some of the possible categories have no member microbes, with a maximum of three appearing in some categories.

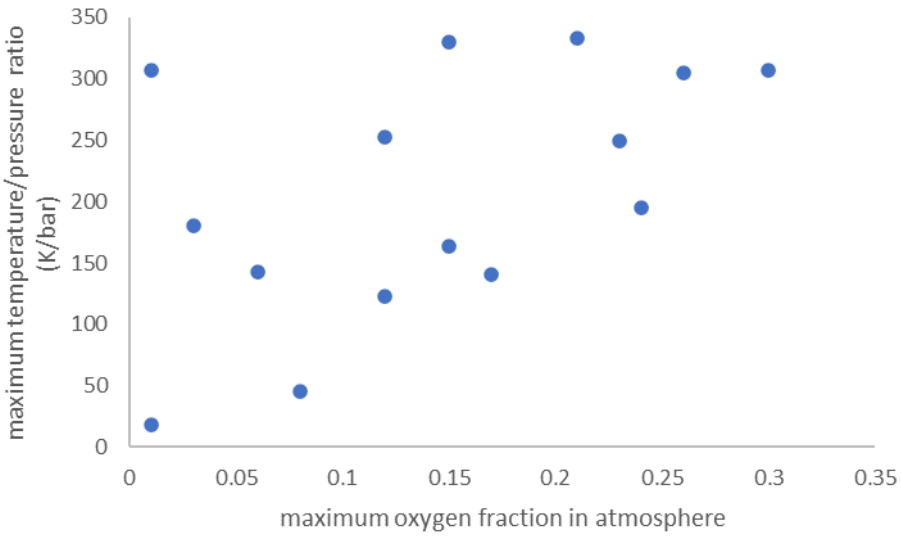


Figure A-2: A plot of two traits: maximum temperature and pressure vs maximum fraction of atmospheric oxygen.

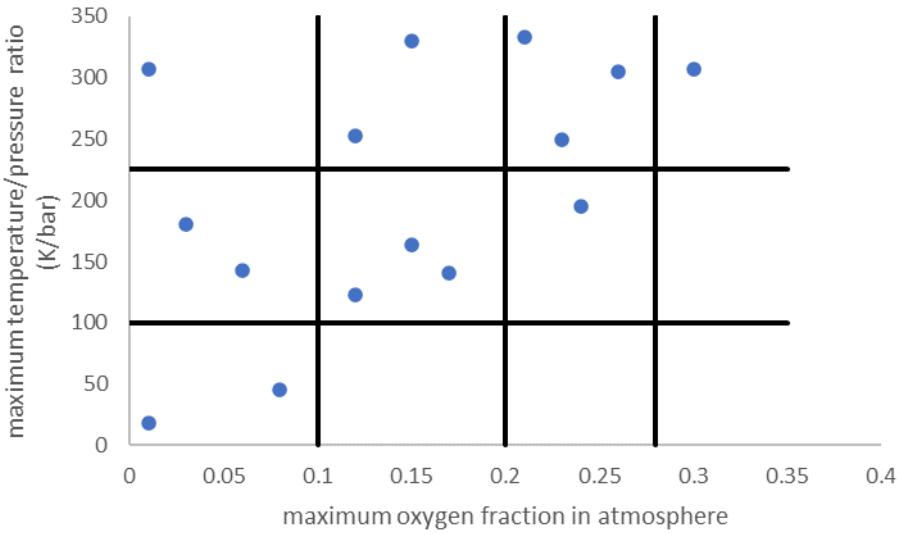


Figure A-3: A matrix of two traits: maximum temperature and pressure vs maximum fraction of atmospheric oxygen divided into 12 categories.

For each of the categories we need to estimate values for key properties that allow us to calculate the probability of having viable microbes when a spacecraft reaches another world. The key properties need to be identified by suitable experts. An example can be: "the expected maximum temperature that a microbe can survive". It is unlikely that we will be able to define such a temperature exactly. There is always some uncertainty, but it is usually possible to place upper and lower bounds on the value. Experiments can help reduce the uncertainty and Bayesian statistics can help us combine experimental data with expert opinion. Some experiments can be able to target a particular class of microbe, while others can cover multiple classes. Bayesian statistics can handle this situation and allow us to refine the uncertainty on the individual classes.

A multidimensional mathematical space can be created onto which the properties of microbes can be plotted. The properties are assigned by experts and can involve uncertainty that can be accommodated by the incorporation of upper and lower bounds.

Once the key properties have been estimated then we can calculate the probability of survival for a specific mission. If the maximum temperature that can be experienced is known, then we can calculate the probability that a microbe in a particular class can survive. We also need an estimate of the number of microbes from each class that can be on the spacecraft.

Almost none of the numbers that we need can be precisely known and there is uncertainty in everything. Yet, the advantages of this approach are: i) all relevant data can be represented on the plot and allocated to categories and ii) statistical modelling can handle all of the uncertainties.

To create the classification system, each of the metrics is split into intervals. For instance, for the "Maximum UV-C (J/m^2) in any media that can be survived" metric can be split into the following intervals:

- 0 to 10.
- 11 to 100.
- 101 to 500..
- 501 to 1000.

- 1001 to 2000.
- 2001 to 10000.

A microbe is a potential member of an interval until experimental data rules it out from the interval. Using the list of traits generated from the data supplied by Dr Petra Rettberg a category can be defined by Table A-1

Table A-1: Parameters that can be used for classification

Minimum growth temperature on any medium	- 9 to +0
Maximum growth temperature on any medium	+ 41 to +100
Maximum survival temperature on any medium in any state	+ 41 to +100
Minimum active water fraction that allows growth	0,40 to 0,49
Maximum number of Freeze-thaw cycles that can be survived	2 to 5
Maximum number of days at -80C that can be survived	6 to 15
Maximum number of days of desiccation that can be survived	6 to 15
Maximum UV-C (J/m^2) in any media that can be survived	1001 to 2000
Maximum polychromatic UV in any media that can be survived	10001 to 20000
Maximum x-ray in any media (Gy) that can be survived	1001 to 2000

In Annex A.3.1 are the membership data for 10 microbes across the 10 proposed metrics.

Microbe properties (e.g. maximum growth temperature) can be expressed as values and each of the range of values (metrics) can be split into intervals. The intervals define the boundaries of categories, and all microbes can be assigned to at least one of the categories.

Having identified the different categories that cover the mathematical space defined by the metrics we need to calculate the survival probability for microbes in each category. The survival probability is the product of three component probabilities: the probability that a microbe survives the planned journey of the spacecraft; the probability that the spacecraft suffers an unplanned catastrophic descent to the planet/moon; and the probability that a microbe survives this unplanned descent. For any microbe that do reach the surface in a viable state we need to calculate the probability that they can migrate into a body of liquid water where they can proliferate. Some of these probabilities can be calculated by comparing the environmental conditions that microbes can be exposed to with the traits of each category. The probability of an unplanned descent is the subject of a different mathematical analysis.

The probabilities of survival for microbes in each category can be calculated and is the product of several probabilities relevant to the situation and/or conditions in question.

A.2.2 Unplanned Descent

To quantify the probability of an unplanned descent we need to consider the possible final states for a spacecraft and the sequence of events that lead to each state. There are several final states for the spacecraft:

- Mission completed and spacecraft disposed of with zero probability of contamination.
- Spacecraft lost with zero probability of contamination.
- Multiple variants of the spacecraft being distributed over multiple impact sites some of which are contaminated.

Each of the variants that results in an impact can be potentially different because they have a different history. This history is characterised by the different expectation and variance, due to uncertainty, for the angle and velocity of any impact with the surface.

A.2.3 Calculating the probability of contamination

Given the independence of the events that need to occur for contamination to happen, we can separate the analysis into at least two parts: the events that lead to an impact; and the events post impact.

If we follow the spacecraft through time, then there is a probability that it never reaches the target world. If it does reach the world for its first fly-by then we have four possible outcomes. In the literature [RD31] these are described as:

1. Continuing the planned tour (no impact).
2. Potential to replan the tour (no impact).
3. Loss of mission (no impact).
4. Impact.

For any possible fly by we have these same four possible outcomes, which can be visualised as a simple tree as shown in Figure A-4.

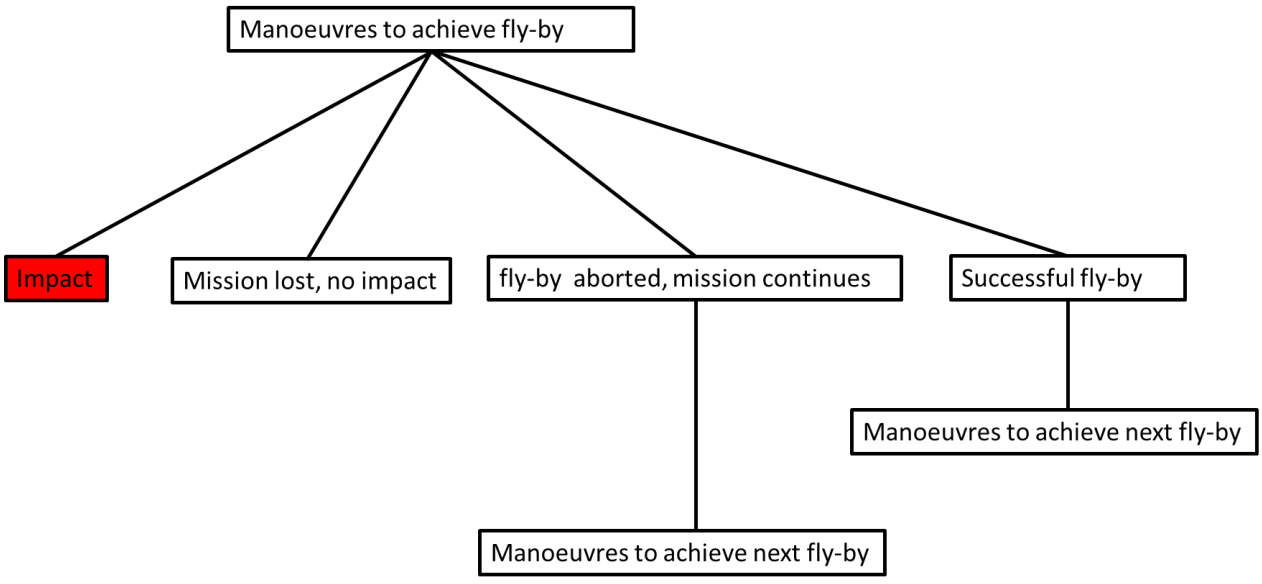


Figure A-4: The four possible outcomes of an attempted fly-by.

We can therefore build up a complex tree which captures all the possible outcomes of multiple fly-bys. In Figure A-5 we illustrate the top of the tree for the first three fly-bys. Each of the red rectangles represents a different impact event.

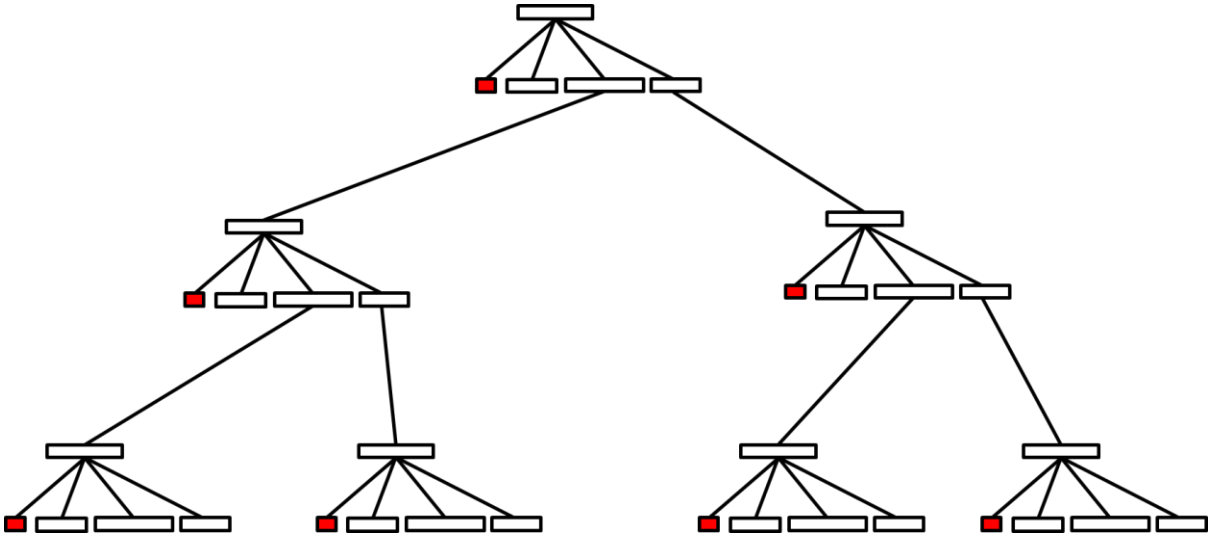


Figure A-5: A tree representing the outcomes of the first three fly-bys.

In total for JUICE there are planned to be 40 fly-bys (Smith and Hendrickson 2022), therefore the number of different impact events in the tree is

$$2^{40} - 1 \approx 1{,}1 \times 10^{12}$$

This number is sufficiently large that we cannot reasonably expect to calculate the probability of contamination arising for each of the cases. It is important to make some simplifying assumptions that can make the total calculation tractable.

Assumption: If we assume that any impact event does not depend on the history of previous successful or unsuccessful fly-bys, but only on the target for that fly-by then all the impact events at any level within the tree are identical. A consequence of this is that we do not need to differentiate between successful fly-bys and those unsuccessful fly-bys that result in the tour needing to be replanned to achieve the next fly-by objective. This means we can collapse the tree so that there is just one impact event at each level, as illustrated in Figure A-6.

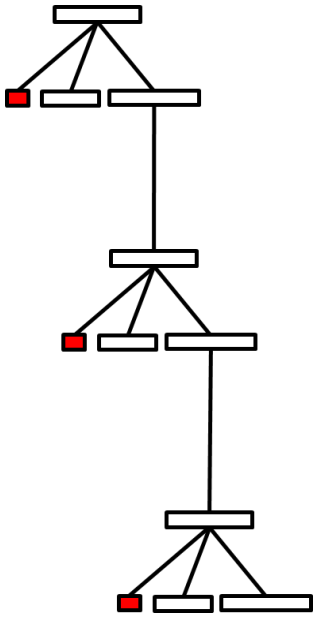


Figure A-6: The collapsed tree, for the first three fly-bys, when the impacts at every level of the original tree are considered as identical events.

This reduces the number of impact events to be considered to 40. The probabilities of impacts and mission continuation can be different for each fly-by. The loss of the detail within the tree can to some extent be compensated for by increasing the uncertainty, i.e. the variance, associated with the angle and velocity of any impact.

The probability of an impact can be expressed by the equation:

$$P(I) = P(r)\left\{\sum_{i=2}^{40}\left\{P(f_i)\prod_{j=1}^{i-1}P(s_j)\right\} + P(f_1)\right\}$$

where

- $\boldsymbol{P(I)}$ is the probability that an impact occurs on Europa at some point in the mission.
- $\boldsymbol{P(r)}$ is the probability that the space craft reaches Europa in a functioning state.
- $\boldsymbol{P(f_i)}$ is the probability that the space craft crashes on the *i*-th fly-by given that it has survived all the previous fly-bys in an operational state.
- $\boldsymbol{P(s_i)}$ is the probability that the space craft survives the *i*-th fly-by in an operational state given that it has survived all the previous fly-bys in an operational state.

It can be noted that for each fly-by there is a third probability $P(l_i)$, which is the probability that the mission is lost on the i-th fly-by given that it has survived all the previous fly-bys in an operational state. Given that there are just the three possible outcomes for each fly-by we have

$$P(f_i) + P(l_i) + P(s_i) = 1$$

If $P(I)$ was to be less that the target probability of 1×10^{-4} then there is no need to consider the survivability of any impact or the possibility of any microbe being transported to a liquid water body on Europa.

It is unlikely that we can be able to place values on the probabilities $P(f_i)$ and $P(s_i)$ with absolute certainty, therefore each probability can have an associated probability distribution. This uncertainty is needed to be included via a Monte-Carlo integration.

A.2.4 Modelling contamination

If the probability of there being an impact is greater than 1×10^{-4} then we need to consider the possibility that an impact can result in the contamination of a liquid water body on an Icy World, i.e. Europa. The equation for the probability of an impact can be modified to give

$$P(C) = P(r)\left\{\sum_{i=2}^{40}\left\{P(f_i)P(c_i)\prod_{j=1}^{i-1}P(s_j)\right\} + P(f_1)P(c_1)\right\}$$

where

- $\boldsymbol{P(C)}$ is the probability that Europa is contaminated by the mission.
- $\boldsymbol{P(c_i)}$ is the probability that contamination occurs given that the *i*-th fly-by resulted in an impact.

All possible impacts can be modelled within a single framework. At the point when a fly-by fails a spacecraft can have one or more viable microbes. During decent to the surface the spacecraft can partially disintegrate and as parts of the spacecraft hit the surface, we can get further disintegration. This can result in one or more impact sites, some of which can be contaminated with viable microbes. We then need to consider whether any microbes at a site can be transported to a body of liquid water and then grow.

Given that the number of microbes, the number of impact sites and the transportation of the microbes are independent of each other there are multiple routes to model the situation that can obtain the same answer. The routes can have different computational burdens, which can make one route preferable.

One route can be to calculate the number of impact sites, each impact site can then be assumed to have an equal probability of being contaminated and an equal probability of any microbes contaminating a body of liquid water. Another route is to assume that we have only a small number of microbes and that we need to track where these go when they hit the surface. The simplest assumption is that there is either one impact or that the number of contaminated impact sites is equal to the number of microbes. Which of those two states we can be in can depend on the velocity and angle of impact. In modelling described elsewhere [RD 31] if the angle between the surface and the direction of travel is greater than approximately 15° then there will be only one impact site, for angle less than this there will be multiple impact sites which we can take to be larger than the number of microbes present.

There are multiple routes to take when modelling the contamination of subsurface liquid water following impact on an icy world. The computational burned for each route will be different and decisions need to be taken based on resource availability.

Surface Contamination and Transportation

To calculate the probability that a viable microbe on the surface can be transported into liquid water within the 1000-year time frame depends on our understanding of the mechanisms that exist for this to occur. We need to know what mechanisms exist, how frequently they occur, the areal extent of the mechanism and how quickly they can move material from the surface to a liquid water body.

Previous work by [RD31] stated that it is highly unlikely that spacecraft material will be introduced into the subsurface liquid body within 1000 years. Our work indicates that previous assumptions need to be updated. We now understand that subsurface meltwater lenses on icy moons like Europa can occur at depths an order of magnitude less than previously thought. General assumptions that subsurface liquid water occurs at 35 km (0 to 50 km in [RD31] need now be reduced to ~ 3 km with assertions that subsurface meltwater lenses exist.

As the depth of liquid water tends towards zero then the probability that any contamination can reach liquid water within the 1000-year time frame will tend to one. The reduction of the depth of liquid water can be expected to significantly increase the probability of transportation to occur. Very simple statistical models that assume no change in the transport properties suggest that the probability can be as high as 0,4. It is almost certain that the transport properties have changed if these relatively shallower liquid water lenses exist. Therefore, it will be necessary to reassess the likely time scales for transport to occur and the probability of it happening within a 1000-year time scale.

A.2.5 Data integration

We desire that the probability of one or more viable microbes reaching liquid water within 1000 years be less than 10^{-4}. Equivalently we can say that the probability of there being zero viable microbes is greater than 0,9999. The sum of these two complementary outcomes is one. Mathematically these can be written as:

$$P(N = 0 \mid t = 1000\ years) \geq 0{,}9999$$
$$P(N \geq 0 \mid t = 1000\ years) \leq 0{,}0001$$

To obtain these probabilities we first need to produce a state vector for each possible combination of location on the spacecraft and each class of microbe

$$S = \{P(N\ = 0|\ t, C, L), P(N\ = 1|\ t, C, L), P(N\ = 2|\ t, C, L)\ \ldots.\}$$

Where t is time, C is microbe class, and L is the location on the spacecraft. For a given class and location, the first element of the vector will be the probability of there being zero viable microbes of class C at location L. The second element will be the probability of there being exactly one viable microbe. Similarly for the other elements of the state vector.

We are interested in the state vector at four specific times and locations:

- $\boldsymbol{S_0}$ ready for launch.
- $\boldsymbol{S_1}$arrival at target body.
- $\boldsymbol{S_2}$ on the surface of the target body.
- $\boldsymbol{S_3}$ in liquid water 1000 years after launch.

To describe the changes in the state vector between each of these times we can use a Markov matrix

$$S_{i+1} = S_i M_{i,i+1}$$

Where the elements of the Markov matrix $M(p,q)$ give the probability that if you start with q viable microbes then you will finish with r viable microbes. Unless the microbes can multiply then $M(r,q) = 0$ if $r > q$, ie you cannot finish with more microbes that you start with. We also have the condition that

$$\sum_r M(r,q) = 1$$

If we define $P(N\ = 0|C,L)$ to be the first element of the state vector S_3 for the combination C,L then

$$P(N = 0 \mid t = 1000\ years) = \prod_{C,L} P(N\ = 0|C,L)$$

The three Markov matrices $M_{0,1}$, $M_{1,2}$ and $M_{2,3}$depend on the physics that are active during the transition and the ability of the microbes to survive them. There are potentially different matrices for each combination C,L. The initial states will reflect the knowledge about the number of microbes on the spacecraft prior to launch and will depend on measurements made prior to launch.

A.2.6 Example of how microbe data is integrated

To facilitate a better understanding of the process we can describe a limited, non-realistic, example. We can assume that the spacecraft can be treated as a single object and hence that there is a single L. We also assume that there are three classes of microbe A, B and C, and that the maximum number if microbes in the initial state are five.

From measurements in the clean room such as spore counts and metagenomics, we can estimate the initial state vectors. We can assume that the metagenomics indicate that there are no microbes of Class C present. The three initial state vectors are taken to be

$$S_0(A) = \{0.0, 0.0, 0.0, 0.0, 0.1, 0.9\}$$
$$S_0(B) = \{0.0, 0.0, 0.0, 0.1, 0.4, 0.5\}$$
$$S_0(C) = \{1.0, 0.0, 0.0, 0.0, 0.0, 0.0\}$$

So, for the **class A** microbes the probability of there being four microbes present is 0,1 and the probability for there being five microbes is 0,9. For the class B microbes we allow the possibility of three, four or five microbes being present. For the class C microbes, the experiments have determined that there are zero microbes present and hence the first value is 1,0. The sum of the probabilities in any state vector is one.

For the journey to the target, we can assume that class A microbes have a probability of survival of 0,5 (see Table A-2). The following is the state vector $S_0(A)$, the Markov matrix $M_{0,1}$ and the second state vector $S_1(A)$. In the matrix we observe that there are initially zero microbes then with probability one there will be zero microbes after the transition. If we start with one microbe then there is a probability of 0,5 of having zero microbes after the transition and 0,5 of having one microbe. The probabilities follow the binomial distribution. The Markov matrix is independent of the initial state vector. The second state vector is given on the right and we see there are different probabilities for the different numbers of microbes 0-5.

Table A-2: Class A microbes state vectors

0	1	0,5	0,25	0,125	0,0625	0,03125	0,034375
0	0	0,5	0,5	0,375	0,25	0,15625	0,165625
0	0	0	0,25	0,375	0,375	0,3125	0,31875
0	0	0	0	0,125	0,25	0,3125	0,30625
0,1	0	0	0	0	0,0625	0,15625	0,146875
0,9	0	0	0	0	0	0,03125	0,028125

For the **class B** microbes, we have a different initial state vector that represents a different opinion on the number of microbes present, and it is assumed that a microbe has a probability of survival of 0,6. This results in a different second state vector (see Table A-3).

Table A-3: Class B microbes state vectors

0	1	0,4	0,16	0,064	0,0256	0,01024	0,02176
0	0	0,6	0,48	0,288	0,1536	0,0768	0,12864
0	0	0	0,36	0,432	0,3456	0,2304	0,29664
0,1	0	0	0	0,216	0,3456	0,3456	0,33264
0,4	0	0	0	0	0,1296	0,2592	0,18144
0,5	0	0	0	0	0	0,07776	0,03888

For the **class C** microbes, we know that the initial state is that there are no microbes present, so the first term is set to one and the rest are zero. For the Markov matrix we have assumed that any class C microbes can have a probability of survival of 0,8. The second state vector is the same as the first state vector, i.e. we still have zero class C microbes (see Table A-4).

Table A-4: class C microbes state vectors

1	1	0,2	0,04	0,008	0,0016	0,00032	1
0	0	0,8	0,32	0,096	0,0256	0,0064	0
0	0	0	0,64	0,384	0,1536	0,0512	0
0	0	0	0	0,512	0,4096	0,2048	0
0	0	0	0	0	0,4096	0,4096	0
0	0	0	0	0	0	0,32768	0

For the second transition we assume that there is a probability of 0,9 that no crash onto the planet occurs and that if a crash does occur then microbes of any class have a probability of surviving of 0,5

For **class A** microbes we then have, from which we can see that the probability of zero viable microbes on the surface is over 0,92 (see Table A-5).

Table A-5: Class A microbes

0,034375	1	0,95	0,925	0,9125	0,90625	0,903125	0,924521
0,165625	0	0,05	0,05	0,0375	0,025	0,015625	0,039814
0,31875	0	0	0,025	0,0375	0,0375	0,03125	0,02584
0,30625	0	0	0	0,0125	0,025	0,03125	0,008379
0,146875	0	0	0	0	0,00625	0,015625	0,001357
0,028125	0	0	0	0	0	0,003125	8,79E-05

For the **class B** microbes we have, again the probability of zero microbes on the surface is over 0,92 (see Table A-6).

Table A-6: Class B microbes

0,02176	1	0.,5	0,925	0,9125	0,90625	0,903125	0,921438
0,2864	0	0,05	0,05	0,0375	0,025	0,015625	0,038882
0,29664	0	0	0,025	0,0375	0,0375	0,03125	0,027909
0,33264	0	0	0	0,0125	0,025	0,03125	0,009909
0,18144	0	0	0	0	0,00625	0,015625	0,001742
0,03888	0	0	0	0	0	0,003125	0,000122

For the **class C** microbes we have and there are still zero microbes (see Table A-7).

Table A-7: Class C microbes

1	1	0,95	0,925	0,9125	0,90625	0,903125	1
0	0	0,05	0,05	0,0375	0,025	0,015625	0
0	0	0	0,025	0,0375	0,0375	0,03125	0
0	0	0	0	0,0125	0,025	0,03125	0
0	0	0	0	0	0,00625	0,015625	0
0	0	0	0	0	0	0,003125	0

For the final transition we assume that all classes of microbe have a probability of 0,1 of contaminating a liquid water body. For the **class A** microbes we have, and we can see that the probability of zero microbes is over 0,98 (see Table A-8).

Table A-8: Class A microbes

0,924521	1	0,9	0,81	0,729	0,6561	0,59049	0,988335
0,039814	0	0,1	0,18	0,243	0,2916	0,32805	0,011093
0,02584	0	0	0,01	0,027	0,0486	0,0729	0,000557
0,08379	0	0	0	0,001	0,0036	0,0081	1,4E-05
0,001357	0	0	0	0	0,0001	0,00045	1,75E-07
8,79E-05	0	0	0	0	0	0,00001	8,79E-10

For the **class B** microbes, we have again a probability of over 0,98 of zero microbes (see Table A-9).

Table A-9: Class B microbes

0,921438	1	0,9	0,81	0,729	0,6561	0,59049
0,038882	0	0,1	0,18	0,243	0,2916	0,32805
0,027909	0	0	0,01	0,027	0,0486	0,0729
0,009909	0	0	0	0,001	0,0036	0,0081
0,001742	0	0	0	0	0,0001	0,00045
0,000122	0	0	0	0	0	0,00001

There continues to be zero **class C** microbes (see Table A-10).

Table A-10: Class C microbes

1	1	0,9	0,81	0,729	0,6561	0,59049	1
0	0	0,1	0,18	0,243	0,2916	0,32805	0
0	0	0	0,01	0,027	0,0486	0,0729	0
0	0	0	0	0,001	0,0036	0,0081	0
0	0	0	0	0	0,0001	0,00045	0
0	0	0	0	0	0	0,00001	0

Finally, we can calculate the probability of there being zero microbes of any class

$$P(N = 0 \mid t = 1000\ years) = 0{,}988335 \times 0{,}87475 \times 1 = 0{,}975956$$

So, the probability of there being contamination by at least one microbe is 0,024044.

A.2.7 Application of results to various scenarios

To apply our results to a number of scenarios (e.g. different icy moon targets) we can make a number of assumptions:

- Statistical certainty is elusive, so estimated probabilities range from 0,01 to 0,99.
- We assume that delivery of a microbe to liquid water on the surface of an icy moon has a probability of 0,99.
- We assume that the probability of contamination of subsurface liquid water by a viable organism increases progressively from 0,01 at 35 km to 0,99 at the surface and the change between the two occurs in an exponential fashion.

McCoy et al. (2021) [RD 31] assessed that delivery of a microbe to subsurface liquid water at 35 km depth beneath Europa generated a probability of 0,01 for that depth. Calculations can be performed that generate different probabilities dependent on the variables involved (see Table A-11)

Table A-11: Probability of contamination elements

Item	Definition
P(R)	Probability that spacecraft arrives at the target in a functioning state
P(f)	Probability that whilst attempting a fly-by a crash occurs (only consider a single fly-by, without reference to any others)
P(s)	Probability that the spacecraft survives a fly-by in an operational state (i.e. it can continue to the next fly-by)
P(l)	Probability that the spacecraft is lost on a fly-by but does not crash (this is a check and the number generated is less than 1 and greater than zero)
N	Number of fly-bys to be attempted
P(c)	Probability of surface-subsurface water transfer of live microbe (related to microbe survivability and ice thickness/distance)
P(C)	Probability of contamination

As an example, Table A-12 lists different overall probabilities of contamination. Most variables are constant with only the probability of surface-subsurface water transfer (related to microbe survivability and ice thickness/distance) is varied. The overall probabilities of contamination were calculated using the formula in A.2.4.

Table A-12: probabilities of contamination for different scenarios

Item	Scenario #1	Scenario #2	Scenario #3	Scenario #4	Scenario #5
P(R)	0,9	0,9	0,9	0,9	0,9
P(f)	0,01	0,01	0,01	0,01	0,01
P(s)	0,95	0,95	0,95	0,95	0,95
P(l)	0,04	0,04	0,04	0,04	0,04
N	40	40	40	40	40
P(c)	0,01	0,03	0,05	0,07	0,09
P(C)	0,001568678	0,004706034	0,007843391	0,010980747	0,014118103

Although gross assumptions can be made, the probability of surface-subsurface water transfer related to ice thickness is expected to be non-linear and requires further dedicated work. However, simply varying the numbers illustrates the effects on the overall probability. Once the accurate probabilities of surface-subsurface water transfer have been calculated they can be used with the supplied spreadsheet.

A.2.8 Worked example for the probability model

A.2.8.1 Introduction

To obtain background information, we use metagenomics and phenotypic data to assess survivability of microbes for e.g. on Europa. Using a matrix of all possible responses, microbe species can be grouped based on their characteristics to identify those that have the potential to cause problems on Europa. The initial quantity of microbes on the spacecraft that are in the high survivability group is considered, because the abundance of microbes has an important influence on the probability of eventual survival of at least one microbe at the surface of Europa. We can then calculate the probability of a viable organism surviving the journey to the subsurface liquid water body within 1000 years following an unplanned impact of the spacecraft on the icy moon surface as follows:

A.2.8.2 Probability of a crash

The probability of an impact can be expressed by the equation

$$P(I) = P(r)\left\{\sum_{i=2}^{N}\left\{P(f_i)\prod_{j=1}^{i-1}P(s_j)\right\} + P(f_1)\right\}$$

where

- $\boldsymbol{P(I)}$ is the probability that an impact occurs on Europa at some point in the mission.
- $\boldsymbol{P(r)}$ is the probability that the space craft reaches Europa in a functioning state.
- $\boldsymbol{P(f_i)}$ is the probability that the space craft crashes on the i-th fly-by given that it has survived all the previous fly-bys in an operational state.
- **N** is the number of fly-bys.

- $\boldsymbol{P(s_i)}$ is the probability that the space craft survives the i-th fly-by in an operational state given that it has survived all the previous fly-bys in an operational state.

We assume the following:

- There is a single crash site, i.e. the space craft does not disintegrate during decent and there is no significant bounce following the impact.
- There are N = 2 fly-bys.
- The probability that the space craft arrives in a functioning state is $P(r) = 0{,}95$.
- The probability that the space craft survives an individual fly-by in an operational state is $P(s_i) = 0{,}98$.
- The probability of crashing during an individual fly-by is $P(f_i)$ =0,00008 (8×10^{-5}).

These numbers can be combined to give a probability of the space craft crashing into the target moon of $P(I) = 1{,}5 \times 10^{-4}$

A.2.8.3 Probability of microbial contamination

We assume the following:

- There are two classes of microbe to be considered
- At launch there are 200 microbes of each class on board the space craft
- The probability of survival for the first class is 0,01, and for the second class it is 0,1
- The probability of at least one microbe from the first-class surviving is $1 - (1 - 0{,}01)^{200} = 0{,}866$
- The probability of at least one microbe from the second-class surviving is $1 - (1 - 0{,}10)^{200} = 0{,}999$
- The probability of at least one microbe from either class surviving is $1 - (1 - 0{,}866)(1 - 0{,}999) = 0{,}99987$

A.2.8.4 Probability of subsurface transportation

We consider two cases:

- There is liquid water of at a depth of 3 km and the probability of surface contamination reaching the water within 1000 years is $0{,}793^3 = 0{,}50$
- There is liquid water at a depth of 20 km and the probability of surface contamination reaching the liquid water within 1000 years is $0{,}793^{20} = 0{,}0098$

Final probabilities

The equation for the probability of contamination is

$$P(C) = P(r)\left\{\sum_{i=2}^{40}\left\{P(f_i)P(c_i)\prod_{j=1}^{i-1}P(s_j)\right\} + P(f_1)P(c_1)\right\}$$

where

- $\boldsymbol{P(C)}$ is the probability that Europa is contaminated by the mission.

- $\boldsymbol{P(c_i)}$ is the probability that contamination occurs given that the i-th fly-by resulted in an impact. $P(c_i)$ is the probability that the space craft is contaminated times the probability that any contamination reaches liquid water.

We can then obtain probabilities for our two cases:

- If the ice is at a depth of 3 km, then the probability of contamination occurring within 1000 years is $0{,}752 \times 10^{-4}$.
- If the ice is at a depth of 20 km, then the probability of contamination occurring within 1000 years is $0{,}0147 \times 10^{-4}$.

The findings from this example, along with a newly developed process of combining all data into a probabilistic calculation can enhance our future ability to accurately assess the risks associated with interplanetary contamination.

A.3 Planetary Protection Analysis using CUDAjectory

A.3.1 Background

Planetary Protection policies exist to prevent the contamination of life-enabled environments with external life sources (you do not want to scream *Eureka!* to only later discover that what you found were self-brought bacteria from Earth right?).

Since some of the hardware that we take into space cannot be sterilised (for instance, the launcher's upper stage), our goal is to prevent our trajectories from colliding with the protected bodies in the first place.

Nevertheless, things are complicated by the inevitable uncertainties that real-world spaceflight introduces in our trajectories:

- For launch phases, we need to work with the natural orbit injection inaccuracy of launchers;
- More in general, we can only know the orbit of our spacecraft that comes from orbit determination (OD) processes: however precisely we run OD campaigns, there can always be at least some uncertainty attached to the orbit measurement.

Thus, determining the Planetary Protection compliance of a given mission results in ensuring that the collision probability that stems from such uncertainty sources remains below a case-specific threshold, for all mission phases. Given CUDAjectory's parallelization capabilities, large Monte Carlo simulations are the perfect example where we can make full use of this computational power, producing reliable and accurate collision probability estimates.

A.3.2 Configuring CUDAjectory for Backward Planetary Protection - MSR/ERO-like analysis

First of all, we need to set up CUDAjectory so that it can run a Planetary-Protection Meaningful case. Let's tackle together an example that stems from the Backward Planetary Protection Analysis for the Mars Sample Return - Earth Return Orbiter mission. Being a backward analysis, this time we want to ensure that we are not contaminating *Earth* with possible traces of life from *Mars*.

import cudajectopy as cudaj

```
# We leverage Python's dictionaries to set up the execution and integration parameters.
# This is only the control/numerical part and we can leave it as default for now.
# In case you wanted to play with these parameters, please refer to the full documentation of the `config` module.
execution = cudaj.config.Execution()
integration = cudaj.config.Integration()
```

Now comes the interesting part! We configure our models of relevant Orbital Dynamics for the MSR-ERO case. In this application, we are starting from outer space and coming back to Earth

```
# we can leverage python dictionaries and lists:
# Earth, Moon, Sun, Mars, and Jupiter are the only relevant celestial bodies for this case
activeBodies = ["Earth", "Moon", "Sun", "MarsBarycenter", "JupiterBarycenter"]
# Now some handle files for the ephemeris and the simulation constants
# Please refer to the configuration documentation and the `brie` package documentation for more details
ephemerisFile = "/local/scratch/amasat/SWDevel/briedata/brie/de430.brie"
constantsFile = "/local/scratch/amasat/SWDevel/briedata/constants/de432.json"
# We can finally build our full Physical Environment Configuration:
envDict = {
  "activeBodies": activeBodies,
  "ephemeris": ephemerisFile,
  "constants": constantsFile
}
# and the environment sub-component
environment = cudaj.config.model.Environment(envDict)
```

Now comes the core of the physical models: the force sources and the collision detection

```
# Point-Mass gravity and Solar Radiation Pressure are the only relevant accelerations for this case
accelerations = cudaj.config.model.Accelerations(["PointGravity", "SRP"])
# We now set up collision events with Earth and Moon, as we need to protect both bodies in this case
events = cudaj.config.model.Events()
# We now configure collisions with earth. This tells CUDAjectory to consider a collision
# when the spacecraft is within 1500 km of Earth's surface (this offset is arbitrary and just for example purposes)
events.collision()["Earth"] = {"offset" : 1500}
# We now configure collisions with moon. This tells CUDAjectory to consider a collision
# when the spacecraft is within 100 km of Moon's surface (this offset is arbitrary and just for example purposes)
events.collision()["Moon"] = {"offset" : 100}
```

This is essentially it! We now assemble everything into the model and then in the full configuration

```
# the full model
model = cudaj.config.Model(accelerations, environment, events)
# and the full configuration
config = cudaj.config.Configuration(execution=execution, integration=integration, model=model)
```

A.3.3 Monte-Carlo strategy: preparing the samples from our uncertainty distribution

As we perform a Monte-Carlo simulation, we draw as many samples as we need to assess the compliance with Planetary Protection Requirements. For the Earth's Backward Planetary Protection, we need to ensure that the contamination probability is lower than 1 in 1 Million (i.e.), for 100 years from the beginning of the missions. Out of simplicity, we use this 1 in 1 Million number as the value to validate against. In real world scenarios, a higher collision probability can be tolerated, as other factors concur in the final contamination probability number, including at least:

- Spacecraft failure probability
- Actual contamination probability with the case-dependent bio-burden on the surface of our spacecraft

Let's say that the return condition as measured from the Orbit determination process is given by the following epoch, Cartesian Mean (position and velocity vector), and Covariance, given with respect to Earth and in the ICRF inertial frame:

```
# CUDAjectory reads epochs given in Mean Julian Days from 01/01/2000 00:0000 TDB (MJD2000).
# The given MJD2000 corresponds to 27th November 2035 at 00:00:00.000000 TDB
epoch = 13114.0 # MJD2000
# The Cartesian state is given in km and km/s in the ICRF inertial frame, centered at Earth
mean = [364646.68966499035,
   -264793.64980213024,
   -115659.59147967656,
   0.38684283480214837,
   0.4994279500652708,
   0.1540700419143419]
# The covariance is given in km^2 and (km/s)^2 units, in the ICRF inertial frame, centered at Earth
covariance =[[3.7035682774414993, -4.265729912062015, -1.7410202000573058,
  -1.3961164847122812e-05, 1.3496302380570769e-05, 5.394476816612986e-06],
 [-4.265729912062015, 16.473085472322694, 6.152186114986342,
  8.790954963608879e-06, -5.911604838325765e-05, -2.13705029159942e-05],
 [-1.7410202000573058, 6.152186114986342, 2.3559823682452086,
  3.992326328743088e-06, -2.1981856315385013e-05, -7.969054208262083e-06],
 [-1.3961164847122812e-05, 8.790954963608879e-06, 3.992326328743088e-06,
  1.5925421255471173e-10, 2.4448243012366923e-11, 9.37533359057955e-12],
```

[1.3496302380570769e-05, -5.911604838325765e-05, -2.1981856315385013e-05,
2.4448243012366923e-11, 2.4277265631990007e-10, 8.871769780063317e-11],
[5.394476816612986e-06, -2.13705029159942e-05, -7.969054208262083e-06,
9.37533359057955e-12, 8.871769780063317e-11, 3.451264410229522e-11]]

We can leverage the numpy.random module to draw the samples:

```
import numpy as np

# NOTE: in this example we only draw 1000 samples for demonstration purposes.
# (we are testing this case with a non-powerful machine and we want to keep the computation time low)
# In a real Planetary Protection analysis, you need to draw ~5,000,000 samples
# to be able to robustly assess compliance with a 1 in 1 Million requirement.

nSamples = int(1e3)

random_states = np.random.multivariate_normal(mean, covariance, size=nSamples)
```

We can now proceed with the finalization of the simulation samples:

```
epochs = cudaj.samples.EpochCollection(size=nSamples, value=epoch)
centres = cudaj.samples.CentreCollection(size=nSamples, centre="Earth")
states = cudaj.samples.StateCollection(random_states)
samples = cudaj.samples.SampleCollection(states=states,
                epochs=epochs,
                centres=centres)
# And we add the area to mass ratio times reflectivity coefficient for SRP calculations (in km^2/kg)
samples.ams(cudaj.samples.AMSCollection([6.546406750072738e-08] * nSamples))
```

A.3.4 The Monte Carlo analysis

We can finally create our Monte Carlo Simulation:

```
sim = cudaj.simulation.device.Simulation(config, samples)
```

We are ready to run! Let's assume that this state is given 7 years after the start of the mission, this means that our simulation needs to run for .

```
# CUDAjectory reads time in days, so 93 years correspond to:
years = 93 * 365.25

# And now we run and extract the results:
results = sim.span(years).run().results()[-1]
```

results is a fully functional cudaj.samples.SampleCollection object that contains the samples at the end of the simulation. We can leverage their built-in functionality to query the simulation outcomes:

```
# this gives us a list of booleans indicating which samples ended in collision
collisions = results.status().eventTerminated(what="Collision")
# we can refine the query to check for collisions with Earth and Moon separately
earthCollisions = results.status().eventTerminated(what="Collision", why="Earth")
moonCollisions = results.status().eventTerminated(what="Collision", why="Moon")
```

With these lists of booleans, it is now straightforward to compute the collision probabilities:

```
overallCollisionProbability = sum(collisions) / len(collisions)
earthCollisionProbability = sum(earthCollisions) / len(earthCollisions)
moonCollisionProbability = sum(moonCollisions) / len(moonCollisions)
```

In general, the collision probabilities that we compute with this method can be zero (if no collision event is detected). Nevertheless, this can be only a byproduct of the statistical method that we have chosen (i.e. Monte Carlo). We can account for this fact in the way we compute the confidence interval, recalling that they are simple binomial distributions which can also be one-sided:

```
import scipy.special as sciSpe

# Here our customized function for the one/two-sided collision probability confidence intervals
def binomial_confidence_interval(n, k, CL, method='exact'):
    '''For a binomial distribution computes the confidence interval
    (Clopper-Pearson-Interval) from the number of trials and the number of
    sucesses to a given confidence level.
    Inputs description:
    n = Total number of trials in binomial distribution (Number of samples)
    k = Number of sucesses in binomial distribution (Number of collisions)
    CL = Confidence level for which confidence interval to compute
```

```
 Outputs description:
 [pmin,pmax] = Minimum/maximum sucess probability value consistent with (n,k) to the given confidence level'''

 # Available methods definition
 availableMethods = ['exact']

 # Check that method selected is available
 if not(method in availableMethods):
  print('Valid methods are ',availableMethods)
  print('Given method was ', method)
  raise ValueError()

 # Compute the confidence interval
 if method == 'exact':
  if k == 0:
   pmax = 1-sciSpe.betaincinv(n-k,k+1,1.-CL)
   pmin = 0
  else:
   pmax = 1-sciSpe.betaincinv(n-k,k+1,(1.-CL)/2.)
   pmin = 1-sciSpe.betaincinv(n-k+1,k,1.-(1.-CL)/2.)
  # Extreme cases
  if k == 0:
   pmin = 0.
  elif k == n:
   pmax = 1.
 return [pmin,pmax]
```

So, the confidence intervals are:

```
overallCI = binomial_confidence_interval(len(collisions), sum(collisions), 0.95)
earthCI = binomial_confidence_interval(len(earthCollisions), sum(earthCollisions), 0.95)
moonCI = binomial_confidence_interval(len(moonCollisions), sum(moonCollisions), 0.95)
```

Finally, we can print the results of our analysis

```
print(f"Overall Collision Probability: {overallCollisionProbability:.8f} with 95% CI: [{overallCI[0]:.8f}, {overallCI[1]:.8f}]")
print(f"Earth Collision Probability: {earthCollisionProbability:.8f} with 95% CI: [{earthCI[0]:.8f}, {earthCI[1]:.8f}]")
```

print(f"Moon Collision Probability: {moonCollisionProbability:.8f} with 95% CI: [{moonCI[0]:.8f}, {moonCI[1]:.8f}]")

Annex B
Templates

B.1 Overview

The organic contamination for bulk materials template, organic contamination for propulsion systems and/or life science support equipment template and biological inventory template can be used to provide information about the amount and type of organics as applicable (**Category III** missions, for e.g. Icy Worlds, Moon, Mars).

B.2 Organic contamination template for bulk materials

Organic contamination template for bulk materials is presented in Table B-1.

Table B-1: Organic inventory template for bulk materials

Date: <Add information>			***Organic material list***					
BULK MATERIALS			***<Add project name>***			**< Add system/subsystem - if applicable>**		
Item No. < add reference to material list - if applicable >	commercial identification	Chemical nature and type of product	Procurement Supplier Specification	Summary of Processing Parameters	Vacuum thermal bake-out/ Sterilisation / Bioburden Reduction (if applicable)	Use and Location/ Product Tree information	Mass (gr)	Outgassing Reference 1. RML % 2. TML % 3. CVCM %
	Adhesives, coating and varnishes							
Example 10.01	Hysol 9395	2-part Epoxy adhesive	Procurement specification: XYZ or Hysol Datasheet number/year	48 hours room temperature cure	Vacuum thermal bake-out at 100 °C x 72 hrs Sterilisation with dry heat for 6 hours at 125 °C (ambient conditions)	Structure subsystem, bonded joints and head locking Product tree ref.: XYZ or drawing number XYZ	100	RML 0,6 % TML 0,1 % CVCM 0,001 % (Report ref. XYZ)
	Adhesive tapes, film and foils							
Example 11.01	TEMP-R-TAPE K103	KAPTON FILM ACRYLIC ADHESIVE	Datasheet	3 hours at 120 °C	Sterilisation with dry heat	Structure THERMAL CONTROL TAPE	20	RML 0,2 % TML 0,1 % CVCM 0,0017 % (Report ref. XYZ)

Date: <Add information>			*Organic material list*					
BULK MATERIALS			*<Add project name>*			**< Add system/subsystem - if applicable>**		
					for 6 hours at 12 5°C (ambient conditions)			
	Paints, Primer and Inks							
Example 12.01	SG 12I FD	SILICON/ZINC OXIDE PAINT	MAP	7 days at RT	Sterilisation with dry heat for 37 hours at 125 °C (ambient conditions)	THERMAL CONTROL PAINT	35	RML 0,6 % TML 0,1 % CVCM 0,2% (Report ref. XYZ)
	Lubricants							
Example 13.01	MOLYCOTE 106 SOLID FILM LUBRICANT	MoS2	Datasheet	1 hour at 200°C	Sterilisation with dry heat for 37 hours at 125 °C (ambient conditions)	LUBRICATION OF FASTENERS	15	RML 0 % TML 0,15 % CVCM 0,12 % (Report ref. XYZ)
	Potting compounds, sealant and foams							
Example 14.01	ROHACELL 110WF	PMI FOAM	Procurement specification: XYZ	N/A	Sterilisation with dry heat for 37 hours at 125 °C (ambient conditions)	FILLER FOR SANWICH PANEL	150	RML 0,10 % TML 0,2 % CVCM 0,12 % (Report ref. XYZ)

Date: <Add information>			*Organic material list*					
BULK MATERIALS			*<Add project name>*			**< Add system/subsystem - if applicable>**		
	Reinforced Plastics							
Example 15.01	Woven M40J/EX1515	cyanate ester matrix resin CFRP prepreg	Procurement specification XYZ	3 hours at 120°C	6 hours at 125 °C	Structure panel skins	500	RML 0,07 % TML 0,15 % CVCM 0,04 % (Report ref. XYZ)
	Thermoplastic and Plastic films							
Example 17.01	KAPTON	POLYIMIDE	Datasheet	N/A	6 hours at 125 °C	SOLAR CELL SUBSTRATE INSULATION	150	RML 0,027 % TML 0,5 % CVCM 0,04 % (Report ref. XYZ)
	Miscellaneous non-metallic materials							
Example 20.1	LACING TAPE SUPERGUDE S-18- DPT-H	DACRON FIBRE; SYNTHETIC RUBBER COAT	Datasheet	N/A	6 hours at 125 °C	Harness and Lacing	65	RML 0,06 % TML 0,2 % CVCM 0,27 % (Report ref. XYZ)

B.3 Organic contamination template for propulsion system and/or life science support equipment

Organic contamination template for propulsion system and/or life science support equipment is presented in Table B-2.

Table B-2: Organic inventory template for propulsion system and/or life science support

Date: <Add information>		***Organic material list***					
Propulsion systems / Life support equipment		***<Add project name>***					**< Add system/subsystem - if applicable>**
Type, i.e. engine type, quantity of engines, size, thrust	Chemical species identification	Chemical species classification	Spacecraft total dry mass (Kg)	Quantity at orbit insertion (Kg)	Quantity at landing (Kg)	Quantity at end of mission (Kg)	Qualitative description of chemical species / major product reactions
Example, monopropellant or bipropellant, MMH-MON reaction control system, 424N	N_2 H_2O CO^2 CO H_2 NO CH_4 Not available	N_2 (major) H_20(major) CO_2 (major) CO (Major) H_2 (minor) NO (minor) Not available (Traces)	<Add mass info>	<Add mass info>	<Add mass info>	<Add mass info>	CH_4 molecules released from the exhaust plume of the lander fuelled by a bipropellant mixture of MMH and MON^{-3}

B.4 Biological inventory

Biological inventory template is presented in Table B-3.

Table B-3:Biological inventory template

Biological material list				
Date: <Add information>		*<Add project name>*		< Add system/subsystem - if applicable>
Type of mission (i.e. payload, spacecraft or crew mission).	**Type of biological material** (i.e. specify if living or dead, , human remains / cremated, ashes, DNA, etc.)	**Brief description about mission/experiment and how biological material is contained**	**Quantity in Kg**	**Information on Disposition** (i.e. waste, remains on Lunar environment)