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**IMPLEMENTING PLANETARY PROTECTION REQUIREMENTS
FOR SPACE FLIGHT**

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DOCUMENT HISTORY LOG

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FOREWORD

This NASA technical standard provides engineering and technical requirements for processes, procedures, practices, and methods that have been endorsed as standard for NASA facilities, programs, and projects, including requirements for selection, application, and design criteria of an item.

This standard establishes the detailed technical requirements to protect and enable current and future scientific investigations by limiting biological and relevant molecular contamination of solar system bodies through exploration activities and protecting the Earth's biosphere by avoiding harmful biological contamination carried by returning spacecraft. This standard provides the technical requirements as an accompaniment to the detailed procedural requirements in NPR 8715.24 Planetary Protection Provisions for Robotic Extraterrestrial Missions and NID 8715.129 Biological Planetary Protection for Human Missions to Mars.

This standard was developed by the NASA Office of Safety and Mission Assurance (OSMA). Requests for information, corrections, or additions to this standard should be submitted to the OSMA by email to Agency-SMA-Policy-Feedback@mail.nasa.gov or via the "Email Feedback" link at <https://standards.nasa.gov>.

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Approval Date

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IMPLEMENTING PLANETARY PROTECTION REQUIREMENTS FOR SPACE FLIGHT

1. SCOPE

1.1 Purpose

1.1.1 The purpose of this standard is to provide technical requirements to protect and enable current and future scientific investigations by limiting biological and relevant molecular contamination of solar system bodies through exploration activities and protecting the Earth’s biosphere by avoiding harmful biological contamination carried on returning spacecraft. The main strategies are to:

- a. Understand and control harmful contamination of other worlds by terrestrial organisms, organic materials, and organic volatile materials carried or released by spacecraft (referred to as forward contamination) in order to assure integrity in the search for evidence of extraterrestrial life and the study of prebiotic chemistry in the solar system for the appropriate period of biological exploration.
- b. Prevent harmful biological contamination of the Earth-Moon system by potential extraterrestrial life and bioactive molecules in returned samples and spacecraft from a sensitive solar system body (referred to as backward contamination).

Note: See Figure 1-1 for a breakdown of the top level planetary protection objective for controlling forward contamination and preventing backward contamination strategies.

PP Objectives Hierarchy – Top Level

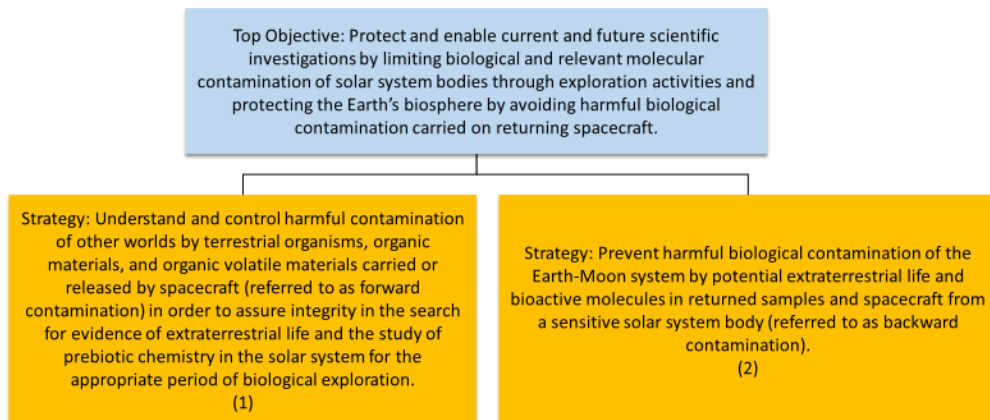


Figure 1-1. PP Top-Level Objective

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1.2 Applicability

1.2.1 This standard is applicable to all missions that may encounter solar system bodies in nominal and credible off-nominal scenarios, including those returning extraterrestrial samples to the Earth-Moon system. Credible off-nominal scenarios include those that could affect compliance with the probabilistic constraints informed by the Committee on Space Research (COSPAR) Planetary Protection Policy and Guidelines released in 2021 (hereafter referred to as COSPAR policy and guidelines).

1.2.2 This standard is approved for use by NASA Headquarters and NASA Centers, including Component Facilities and Technical and Service Support Centers, and may be cited in contract, program, and other Agency documents as a technical requirement. This standard applies to the Jet Propulsion Laboratory or to other contractors, grant recipients, or parties to agreements to the extent specified or referenced in their contracts, grants, or agreements.

1.2.3 In this standard, all mandatory actions (i.e., requirements) are denoted by statements containing the term “shall.” The terms “may” denotes a discretionary privilege or permission, “can” denotes statements of possibility or capability, “should” denotes a good practice and is recommended, but not required, “will” denotes expected outcome, and “are/is” denotes descriptive material.

1.2.4 Projects consult with the Office of Planetary Protection (OPP) to coordinate tailoring considerations for the PP Requirements Document (see Appendix B) based on new knowledge or unique mission configuration (see NPR 8715.24, Planetary Protection Provisions for Robotic Extraterrestrial Missions).

1.2.5 Based on the mission-specific documentation and new knowledge of planetary bodies, revised planetary protection approaches provided in the PP Implementation Plan for missions may be considered by the responsible Mission Directorate and the OPP.

1.2.6 Alternate or novel methods and approaches for planetary protection implementation not included in this standard may be proposed recognizing the need for adequate resources and schedule for review. The OPP verifies the acceptability of all alternative methods prior to adoption by the project based on project-supplied data. In light of current scientific consensus, project-supplied data is examined rigorously by relevant subject matter experts and evaluated for the effectiveness of the alternate method for the specific application under consideration.

1.3 Expected Elements for Deliverables

1.3.1 To demonstrate compliance with the requirements of this standard, projects identify the applicable Agency planetary protection requirements consistent with the planetary protection category, establish their planned implementation, and document the execution of the planned implementation via the deliverables that are required per NPR 8715.24.

1.3.2 The expected elements and the details associated with the accepted provisions, including the accepted set of standards, to populate each of those deliverables are provided in the following appendices:

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- a. Appendix A. PP Mission Categorization Proposal —Expected Elements.
- b. Appendix B. PP Requirements Document—Expected Elements.
- c. Appendix C. PP Implementation Plan—Expected Elements.
- d. Appendix D. Pre-Launch PP Report—Expected Elements.
- e. Appendix E. Post-Launch PP Report—Expected Elements.
- f. Appendix F. PP Extended Mission Report—Expected Elements.
- g. Appendix G. End-of-Mission PP Report—Expected Elements.

1.4 Request for Relief

NPR 8715.3, NASA General Safety Program Requirements, defines the process for tailoring requirements—i.e., requesting and granting relief from requirements—within this standard.

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2. APPLICABLE AND REFERENCE DOCUMENTS

2.1 Applicable Documents

The documents listed in this section contain provisions that constitute requirements of this standard as cited in the text. Use of more recent issues of cited documents may be authorized by the responsible Technical Authority. The applicable documents are accessible via the NASA Technical Standards System at <https://standards.nasa.gov> or may be obtained directly from the Standards Developing Organizations or other document distributors.

2.1.1 Government Documents

NPR 8715.24 Planetary Protection Provisions for Robotic Extraterrestrial Missions

NID 8715.129 Biological Planetary Protection for Human Missions to Mars

2.1.2 Non-Government Documents

ASTM D1193-06(2018) Standard Specification for Reagent Water

ISO 14644-1:2015(E) Cleanrooms and associated controlled environments—
Part 1: Classification of air cleanliness by particle concentration

2.2 Reference Documents

The reference documents listed in this section are not incorporated by reference within this standard, but may provide further clarification and guidance.

2.2.1 Government Documents

NPR 8715.3 NASA General Safety Program Requirements

NASA/SP-2011-3421, Second Edition, Probabilistic Risk Assessment Procedures Guide for NASA Managers and Practitioners, December 2011.

NASA/SP-2011-3422, Version 1.0, NASA Risk Management Handbook, November 2011.

2.2.2 Non-Government Documents

AAMI TIR14:2009 Contract Sterilization Using Ethylene Oxide

AAMI TIR17:2008 Compatibility Of Materials Subject To Sterilization

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AAMI TIR28:2009	Product Adoption And Process Equivalence For Ethylene Oxide Sterilization
AAMI TIR29:2002	Guide For Process Control In Radiation Sterilization
AAMI TIR35:2006	Sterilization Of Health Care Products - Radiation Sterilization - Alternative Sampling Plans For Verification Dose Experiments And Sterilization Dose Audits
AAMI TIR37:2007	Sterilization Of Health Care Products -- Radiation -- Guidance On Sterilization Of Human Tissue-Based Products
AAMI TIR52:2014	Environmental Monitoring For Terminally Sterilized Healthcare Products
AAMI TIR 16775	Packaging for terminally sterilized medical devices — Guidance on the application of ISO 11607-1 and ISO 11607-2
AIA/NAS412 2nd Edition 2018	Foreign Object Damage (FOD) Prevention Guidance Document
ANSI/AAMI/ISO 10993-1: 2018	Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process
ANSI/AAMI/ISO 11607-2:2019	Packaging For Terminally Sterilized Medical Devices - Part 2: Validation Requirements For Forming, Sealing And Assembly Processes
ANSI/AAMI/ISO TIR16775:2014	Packaging For Terminally Sterilized Medical Devices - Guidance On The Application Of ISO 11607-1 And ISO 11607-2
ANSI/AIAA S-120A-2015 (R2019)	Standard: Mass Properties Control for Space Systems
ASTM D3951	Standard Practice for Commercial Packaging
Committee on Space Research (COSPAR)	Planetary Protection Policy, (2021), Space Research Today. 211:12-25.
ECSS-Q-ST-70-01C	Cleanliness and contamination control (15 November 2008)
ECSS-Q-ST-70-50C	Particles contamination monitoring for spacecraft systems and cleanrooms (4 October 2011)

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ECSS-Q-ST-70-53C	Materials and hardware compatibility tests for sterilization processes (15 November 2008)
ECSS-Q-ST-70-54C	Ultracleaning of flight hardware (15 February 2017)
ECSS-Q-ST-70-55C	Microbial examination of flight hardware and cleanrooms (15 November 2008)
ECSS-Q-ST-70-56C	Vapour phase bioburden reduction for flight hardware (30 August 2013)
ECSS-Q-ST-70-57C	Dry heat bioburden reduction for flight hardware (30 August 2013)
ECSS-Q-ST-70-58C	Bioburden control of cleanrooms (15 November 2008)
ECSS-U-ST-20C	Space sustainability – Planetary protection (1 August 2019)
IEST-RP-CC018.4	Cleanroom Housekeeping-Operating and Monitoring Procedures
IEST-RP-CC023.2	Microorganisms in Cleanrooms
IEST-RP-CC027.2	Personnel in Cleanrooms
IEST-STD-CC1246E	Product cleanliness levels – applications, requirements, and determination
ISO 10993-12:2012	Biological evaluation of medical devices - Part 12: Sample preparation and reference materials
ISO 11138-1:2017	Sterilization of health care products - Biological indicators - Part 1: General requirements
ISO 11138-7:2019-03	Sterilization of health care products – Biological indicators – Part 7: Guidance for the selection, use, and interpretation of results.
ISO 11139:2018	Sterilization of health care products — Vocabulary of terms used in sterilization and related equipment and process standards
ISO 11231:2019	Space systems – Probabilistic risk assessment (PRA)
ISO 11607-1:2019	Packaging for terminally sterilized medical devices — Part 1: Requirements for materials, sterile barrier systems and packaging systems

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- ISO 11737-1:2018 Sterilization of health care products - Microbiological methods - Part 1: Determination of a population of microorganisms on products
- ISO 14644-5:2004 Cleanrooms and associated controlled environments – Part 5: Operations
- ISO 14698-1:2003 Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods
- ISO 15026-2 Systems and Software Engineering — Systems and software assurance — Part 2: Assurance case
- ISO/IEC/IEEE 15026-1:2019 Systems and software engineering — Systems and software assurance — Part 1: Concepts and vocabulary
- Kminek, G., et al., (2022) COSPAR Sample Safety Assessment Framework (SSAF). *Astrobiology* vol. 22,S1:S186-S216.
- Kminek, G., et al., (2010) Report of the COSPAR Mars Special Regions Colloquium. *Adv Space Res* 46:811–829.
- Rummel, J.D., et al., (2014) A new analysis of Mars “Special Regions”: findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology* 14:887–968.
- World Health Organization. Medical device regulations: global overview and guiding principles. Geneva, Switzerland. 2003.

2.3 Order of Precedence

2.3.1 The requirements established in this standard do not supersede or waive established Agency requirements found in other documentation.

2.3.2 Conflicts between this standard and other requirement documents shall be resolved by the responsible Technical Authority.

3. ACRONYMS AND DEFINITIONS

3.1 Acronyms and Abbreviations

COSPAR	Committee on Space Research
EDL	Entry, Descent, and Landing
EOM	End of Mission
FO	Foreign Object
FOD	Foreign Object Damage
FOd	Foreign Object Debris
FRR	Flight Readiness Review
HEPA	High-Efficiency Particulate Air
LRR	Launch Readiness Review
OPP	Office of Planetary Protection
OSMA	Office of Safety and Mission Assurance
PPEL	Planetary Protection Equipment List
PPO	Planetary Protection Officer
PSR	Permanently Shadowed Region
RSL	Recurrent Slope Lineae
SRF	Sample Receiving Facility
SRR	System Requirements Review
TSA	Tryptic Soy Agar

3.2 Definitions

Anomaly. An incident or event where observations, data, or analysis results are unexpected, not understood, or could be a potential threat to compliance with planetary protection requirements.

Assay. Collection and analysis of biological contamination with a specified procedure.

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Assurance Case. A reasoned, auditable artifact that supports the contention that its top-level claim (or set of claims) is satisfied, including systematic argumentation and its underlying evidence and explicit assumptions that support the claim(s).

Backward contamination. Extraterrestrial harmful contamination that could pose a threat to the Earth's biosphere.

Bioactive Molecules. Biologically produced catalytic molecules that propagate by utilizing or altering terrestrial biological molecules, structures, or systems and could be a potential threat to the Earth's biosphere, including prions, viruses, gene-transfer agents, and other non-cellular molecular entities.

Bioburden Accounting. Numerical value applied to a viable count to compensate for incomplete removal of microorganisms from a spacecraft and/or failure to culture microorganisms.

Bioburden. Population of viable organisms on or in spacecraft materials.

Biological Contamination. Unwanted presence of biologically produced molecules (including microorganisms) carried by spacecraft hardware, including instruments and experiments, that could either compromise understanding extraterrestrial environments, or present a threat to the Earth-Moon system upon return.

Biological Inoculation Event. Introduction of a viable organism to a solar system body capable of providing nutrients and environmental growth conditions such that the organism can replicate.

Break the Chain of Contact. Prevent transfer to the Earth-Moon System of all unsterilized material of concern originating from another habitable world by ensuring that no hardware that has touched the other world can physically interact with the Earth-Moon system unless that material is robustly contained or demonstrated to be safe for release into Earth's biosphere.

Chemical Evolution. Process of transition from the initial state of the solar system to the present state, particularly with respect to the development of organic molecules and the formation of life.

Cleanroom, Biologically Controlled. Room within which the facility procedures and operations are designed for biological contamination prevention and awareness, including cleaning and monitoring for levels of biological contamination by use of full body coverall, hood, face mask, gloves and boots and restricted access.

Cleanroom, Particle Controlled. Room within which the number concentration of airborne particles is controlled and classified, and which is designed, constructed and operated in a manner to control the introduction, generation and retention of particles inside the room. The degree to which particles are controlled and classified is further defined in ISO-14644.

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Containment. The act, process, or means of keeping bioactive molecules and viable organisms within defined physical limits.

Contamination. Unwanted material present on or in the spacecraft/spacecraft assembly environment or introduced into the environment of a solar system body.

Credible Off-Nominal Scenario. These scenarios cover cases where some condition could occur that results in the system performing in a way that is different from normal and that would affect compliance with the probabilistic constraints informed by current COSPAR policy and guidelines. This includes failures, low performance, unexpected environmental conditions, or operator errors that would affect compliance with the probabilistic constraints informed by current COSPAR policy and guidelines.

Culture-based Assay. An assay involving the collection and growth of viable organism contamination using a specified laboratory procedure, with assessment of accumulation of biomass over time as the assay detection endpoint.

Earth-Moon System. The Earth and the Moon (including artificial objects in orbit around either body) is considered as a single environment for planetary protection purposes in considering sample return from restricted sample return bodies (Mars, Europa, Enceladus, others to be determined) to protect the unrestricted travel within the system.

Earth's Biosphere. Habitable (inhabited or uninhabited) environments of the Earth.

Encapsulated Bioburden. Bioburden within bulk non-metallic spacecraft materials. Examples are bioburden inside paints, conformal coatings, thermal coatings, adhesives, composite materials, closed-cell foam, bulk liquids and bulk gasses.

Essential Personnel. Personnel who conduct a range of operations and services that are typically vital to continue critical operations, as defined in project-level documentation.

Extraterrestrial Life. Metabolically active entity, capable of propagating from outside the Earth's biosphere.

Foreign Object (FO). A substance or article (examples include, but are not limited to: tools, consumables, hardware, product protective devices, personal items, product process debris, operations debris, environmental debris) that could potentially enter and/or migrate into/on the product or system becoming Foreign Object Debris (FOD) and potentially cause Foreign Object Damage (FOD), if not removed and controlled.

Foreign Object Damage (FOD). Any damage attributed to Foreign Object Debris (FOD) that can be expressed in physical or economic terms, which could potentially degrade the product or system's required safety and/or performance characteristics.

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Foreign Object Debris (FOD). Any foreign object (FO) that has entered and/or migrated into/on the product or system, and could potentially cause Foreign Object Damage (FOD), if not removed or controlled.

Forward contamination. Terrestrial based harmful contamination present on a spacecraft during exploration activities.

Genomics-based Assessment. An assay involving the collection and analysis of nucleic acid contamination with a specified procedure.

Ground Support Equipment. Nonflight equipment, systems, or devices designed and developed for a physical or direct functional interface with flight hardware.

Habitable. An environment where physico-chemical limits (availability of water and biomolecule building blocks, presence of an energy source/redox gradient, and permissive temperature and pH ranges) permit replication of carbon-based living organisms.

Harmful Contamination. Unwanted material on the surface of a solid material, or incorporated into a solid, liquid, or gas that damages the integrity of the study of chemical evolution and the origin of life at another solar system body, or that has negative consequences for humans and Earth's biosphere.

Inadvertent Impact. An impact that was not part of baseline operations that could result in harmful contamination addressing both nominal and credible off-nominal trajectories, including orbital insertion anomalies, EDL anomalies, incomplete breakup and burnup, and decay from orbit.

Life Detection. An investigation performed on a sensitive solar system body, or a sample obtained from such a body, to detect biomarkers of extraterrestrial origin from extinct and extant extraterrestrial life forms (up to and including viable organisms).

Microbial Reduction. The process or processes used to reduce the viable microbial population on an item to an acceptable limit.

Mission Operations. Any activity, experiment, or process performed by the spacecraft in its operational lifetime or anticipated after EOM.

Non-Essential Personnel. Personnel not part of the required workforce but that may have access to hardware or environments, including guests, visitors, security, press, and media events, and may include project personnel not part of the critical operations team.

Organic Materials. All carbon-containing compounds excluding carbides, carbonates, cyanides and simple oxides of carbon (i.e., CO and CO₂).

Planetary Protection Category. Category assigned to reflect the interest and concern that terrestrial contamination can compromise future investigations, and the concern for

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extraterrestrial material being harmful to Earth's biosphere by returning spacecraft. Depends on the target body and mission type.

Period of Biological Exploration. The period of 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 consistent with current COSPAR policy and guidelines.

Restricted Earth-Return. Planetary protection sub-category V for sample return missions from solar system bodies deemed by scientific opinion to have a possibility of harbouring indigenous life forms.

Robust Containment. A strategy of utilizing dissimilar, redundant approaches to achieve an overall containment system that is minimally sensitive to engineering operations, stressful environmental conditions, and credible off-nominal scenarios in use from point-of-collection to containment in a receiving facility on Earth.

Sample. Any intentionally collected or unintentionally adhering physical material (including solids, liquids, and gases) carried by a spacecraft returning to the Earth or Earth-Moon system from other solar system bodies (including samples from the Moon being delivered to the Earth).

Sample Safety Assessment. A defined series of laboratory analyses performed under containment to demonstrate, to a specified degree of assurance, that sample material returned from a restricted Earth return mission is free from extraterrestrial material that could cause harmful contamination to the Earth's biosphere.

Sensitive Icy World. Solar system bodies with surface ice and potential for subsurface oceans, for which there is significant scientific interest relative to the process of chemical evolution and the origins of life and for which scientific opinion provides a significant chance that contamination by a spacecraft can compromise future investigations (currently Europa, Enceladus, others TBD by scientific consensus).

Sensitive Solar System Body. Solar system bodies, including planets and moons, for which there is significant scientific interest relative to the process of chemical evolution and the origins of life and for which scientific opinion provides a significant chance that contamination by a spacecraft can compromise future investigations (currently Mars, Europa, Enceladus, others TBD by scientific consensus).

Solar System Body. A solar system planet, moon, asteroid, comet, or other exploration target for a spacecraft mission.

Special Region. An environment at Mars within which terrestrial organisms are likely to replicate. Any region which is interpreted to have a high potential for the existence of extant Martian life forms is also defined as a Special Region. See section A.1.4.3 for further details.

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Spores (endospores). Robust and metabolically dormant forms produced from actively replicating bacteria as a response to environmental stressors. Heat resistant spores are detected using the NASA Standard Spore Assay as an indicator of spacecraft biological cleanliness.

Sterilization. A process designed to reduce bioburden with the intent to destroy or eliminate all forms of viable organisms to a defined assurance level.

Surface Bioburden. Bioburden present on the external and internal surfaces, including surfaces free for gas exchange and surfaces which are sealed from gas exchange.

Terrestrial Organism. Self-propagating or metabolically active cellular system that is from the Earth's biosphere.

Total Bioburden (of a spacecraft or system). A bioburden summation of exposed surfaces, mated surfaces, and encapsulated volumes. Inputs can include direct bioassay data, microbial reduction process credits, and standard values based on manufacturing environment cleanliness.

Viable Organism. An organism that can survive and has the capability of replicating when growth conditions are favorable. A viable organism does not require a host and has an intact cellular membrane.

Volatile Organic Materials. Organic chemicals that are liberated from an organic material into an environment of concern through thermodynamic processes.

Witness Plate. A surrogate, representative spacecraft material that can be utilized as a test article to determine bioburden. The intent is to capture hardware processing during assembly, integration and testing and should be exposed to the identical environment and orientation of the flight hardware.

4. CONTROL OF FORWARD CONTAMINATION

4.1 Purpose

This chapter provides requirements to understand and control harmful contamination of other solar system bodies by terrestrial organisms, and organic materials, and organic volatile materials carried or released by spacecraft (referred to as forward contamination) in order to assure integrity in the search for and evidence of extraterrestrial life and the study of prebiotic chemistry in the solar system for the appropriate period of biological exploration. This is achieved through understanding the mission science objectives related to the contamination sensitivity of the destination. Acceptable methodologies include prescriptive or performance based (e.g., assurance case) approaches. See Figure 4-1 for an overview of controlling forward contamination.

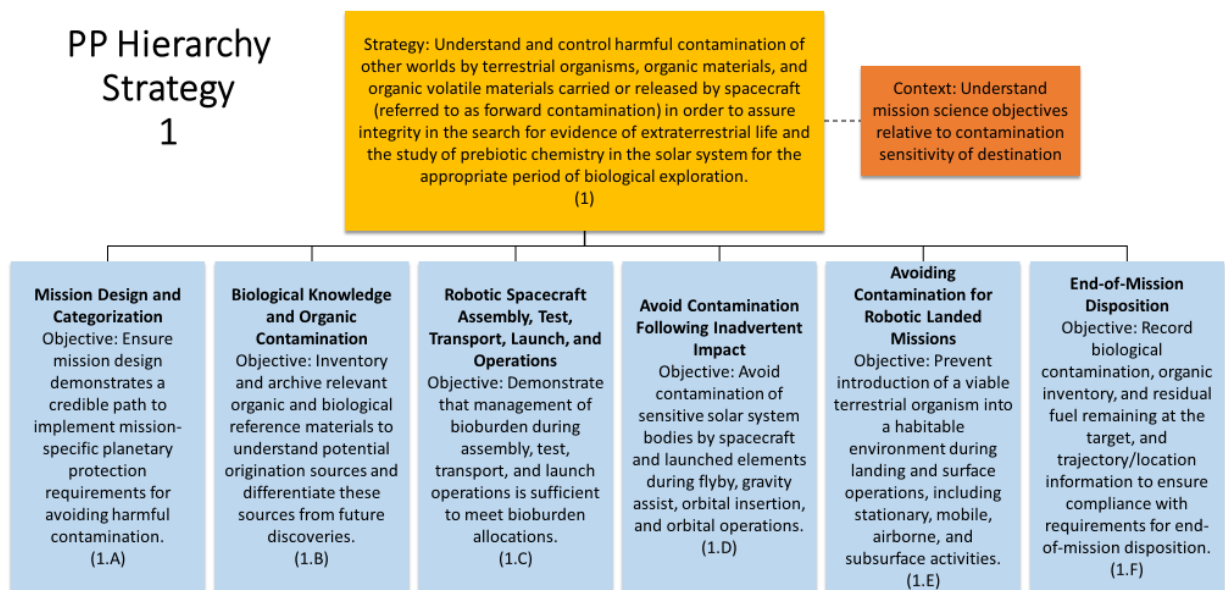


Figure 4-1. PP Strategy 1 – Forward Contamination Control

The entirety of the chapter is applicable to robotic missions. Currently, certain sections are identified as applicable to crewed missions. Crewed mission implementations will be updated as knowledge gaps are closed and policy objectives are finalized.

4.2 Mission Design and Categorization

4.2.1 A planetary protection category reflects the interest and concern that terrestrial contamination can compromise future investigations, and the degree of concern that extraterrestrial material brought by returning spacecraft may be harmful to Earth’s biosphere. Options for categorization of missions to bodies of the solar system are described in NPR 8715.24.

Note: Required elements for Earth-return missions are provided in section 5.2.

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4.2.2 In addition to the required elements specified in NPR 8715.24, the following are to be included in the development of the PP Mission Categorization Proposal.

4.2.2.1 Additional Categorization Elements for Earth's Moon (Category II, IIa, IIb)

4.2.2.1.1 Missions targeting Earth's Moon shall be assigned a final categorization of Category II, IIa, or IIb. For the purposes of planetary protection categorization a Permanently Shadowed Region (PSRs) and the lunar poles are defined as locations south of 79°S latitude and north of 86°N latitude.

4.2.2.1.2 The assignment of specific missions to a planetary protection category is based on the following:

- a. For orbital and flyby missions to Earth's Moon, assignment to Category II.
- b. For missions landing on Earth's Moon, but not a PSR and the lunar poles, assignment to Category IIa.
- c. For missions landing or conducting surface operations on Earth's Moon within a PSR and the lunar poles then the mission shall be assigned Category IIb.

4.2.2.2 Additional Categorization Elements for Ganymede, Titan, Triton, and Pluton/Charon (Category II, III or IV)

4.2.2.2.1 Missions shall be assigned a final categorization of Category II if the remote potential of contamination is demonstrated; otherwise, these missions shall be assigned Category III or IV depending on mission intent.

4.2.2.2.2 The assignment of specific missions to a planetary protection category is based on the following:

- a. Assignment to Category II is supported by an analysis that demonstrates a probability of occurrence less than 1.0×10^{-4} of a biological inoculation event into a potentially habitable aqueous environment within a 1000 year period of biological exploration.

Note: Example parameters of an analysis include ice thickness, presence and abundance of aqueous environments, and plausible contamination pathways at those locations that could lead to a biological inoculation event.

- b. If the less than 1.0×10^{-4} probability of occurrence cannot be supported by analysis alone or if an analysis is not performed, then the mission shall be assigned Category III (orbiter, fly-by) or Category IV (lander).

4.2.2.3 Additional Categorization Elements for Mars (Category IVa, IVb, IVc)

4.2.2.3.1 Category IV missions to Mars are subdivided into subcategories IVa, IVb (life detection goal), and IVc (special region access) as shown in Table 4-1. Missions are

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to achieve compliance with the applicable requirements corresponding to the appropriate IV subcategory. Requirements for missions carrying life detection instruments that access special regions shall combine IVb and IVc requirements, as determined on a mission-by-mission basis.

Note: Life detection may be extant or extinct in nature, while special regions harbor a likelihood for terrestrial organism replication if introduced or a high likelihood of extant life.

Table 4-1. Category IV Mission to Mars Subcategories

		Life detection Goal	
		No	Yes
Special Region Access	No	IVa	IVb
	Yes	IVc	IVb & IVc ¹

¹ Category IV missions to Mars accessing a special region and with a life detection goal are categorized as both IVb and IVc and comply with both sets of requirements.

4.2.2.3.2 Category IVa missions conducting landed operations that are not landing or operating in a special region or conducting life detection do not require any additional categorization elements.

4.2.2.3.3 Category IVb missions conducting life detection investigations, including landed in-situ, remote sensing, or sample return operations shall describe the following:

- a. The nature of investigations to be conducted, including the methods, materials and compounds being targeted, and limits of detection.
- b. An approach to prevent false positive and false negative life detection results. The selected approach may include limits of detection and associated spacecraft cleanliness levels during both pre-launch and science operations, as appropriate.

4.2.2.3.4 Category IVc missions accessing a special region shall identify potential for a biological inoculation event caused by a spacecraft resulting from a nominal or off-nominal operation. Specific cases for special region access are included in Appendix A.

4.2.2.4 Additional Categorization Elements for Europa, Enceladus or Other Sensitive Icy Worlds (Category III or IV)

Category III or IV missions to Europa, Enceladus, or other sensitive icy worlds determined by scientific consensus to be determined, shall identify potential for a biological inoculation event caused by a spacecraft resulting from a nominal or off-nominal operation.

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4.2.2.5 Additional Categorization for Secondary and Auxiliary Payloads (Category II, III, IV or V)

Secondary and auxiliary payloads leaving Earth and having a different target object or mission categorization than the primary payload should address, to the extent known, the following elements:

- a. Identify if separation from the primary payload or launch vehicle is in the baseline mission design concept.
- b. Identify any onboard propulsion system and capabilities.
- c. For payloads targeting a sensitive solar system body, address contamination avoidance at the interface between hardware elements.

Note: Includes secondary and auxiliary payloads being launched from Lagrange points or in halo or Lissajous orbits about Lagrange points.

4.3 Biological Knowledge and Organic Contamination

4.3.1 Planetary missions (lander, probe, orbiter, flyby) shall capture relevant biological and organic contamination knowledge in an inventory and archive relevant organic and biological reference materials from hardware suppliers and processing environments in order to understand future discoveries, as outlined in Table 4-2.

Note: Organic inventory and organic archiving thresholds are informed by current COSPAR policy and guidelines.

4.3.2 Category II, III, & IV missions shall document a formal assessment of organic and biological materials present, as defined in paragraphs 4.3.5 and 4.3.6, in the PP Implementation Plan. Category II & IIa missions to Earth's Moon are excluded from this requirement.

4.3.3 Category IIa & IIb missions to Earth's Moon shall document a formal assessment of volatile organic materials, propellant residuals and combustion products that may be released into the lunar environment by the propulsion, attitude control and other spacecraft systems in the Pre-Launch PP Report.

4.3.4 Category II, III, & IV missions shall document the PP Organic Inventory in the Pre-Launch PP Report.

4.3.5 The PP Organic Inventory shall include all organic materials present on the spacecraft in amounts greater than 1.0 kg.

Note: A complete PP Organic Inventory should include estimates of organic products that may be released into the environment of the sensitive solar system body by propulsion, attitude control and life support systems (if present), and include a quantitative and qualitative description of major chemical constituents and the integrated quantity of minor chemical constituents present.

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Note: A complete PP Organic Inventory should consider contributions from all spacecraft hardware elements including subsidiary payloads that may be present in nominal and credible off-nominal scenarios. Decision on whether subsidiary payload PP Organic Inventory is integrated with or managed separate from the primary payload PP Organic Inventory is determined at SRR or at the point where a rideshare is agreed (see also 4.5.5).

4.3.6 Category III & IV robotic missions shall document the PP Biological Inventory in the Pre-Launch PP Report that includes estimates of all biological materials (intact organisms, remnants of organisms, and biological molecules) intentionally included as part of the hardware elements reaching the target destination (e.g., payload, spacecraft, launch vehicle, descent stage).

Note: This does not include biological materials present on/in the spacecraft as a result of the assembly and integration process.

4.3.7 Category III & IV robotic missions shall archive 50 g of organic materials that are present on the spacecraft in amounts greater than 25 kg.

Note: Archived materials should be collected from those processed for flight (e.g., machined, cured, treated, etc.). Archival should be considered through the end of mission.

4.3.8 Category III & IV robotic missions shall document any archived organic materials, including intended method, duration, and location of archival preservation, in the Pre-Launch PP Report.

4.3.9 Any change to the PP Organic Inventory and the PP Biological Inventory after the delivery of the Pre-Launch PP Report shall be documented in the Post-Launch PP Report and the End of Mission PP Report.

4.3.10 Category IIa & IIb missions to the Moon shall report an inventory of volatile organic materials, propellant residuals and combustion products that remain or have been released into the lunar environment by the propulsion, attitude control and other spacecraft systems in the End of Mission PP Report.

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Table 4-2. Required Mission PP Organic and PP Biological Inventory

	PP Organic Inventory		PP Biological Inventory ^c	Organic Archive ^d
	Propulsion/Attitude Control System Materials ^a	Entire Spacecraft ^b		
Category II (excluding Lunar)	--	Required	--	--
Category II (Lunar)	--	--	--	--
Category IIa (Lunar)	Required	--	--	--
Category IIb (Lunar)	Required	Required	--	--
Category III	--	Required	Required	Required
Category IV	--	Required	Required	Required

a. As defined in paragraph 4.3.3.

b. Organic materials that are present on the spacecraft in amounts greater than the thresholds provided in paragraph 4.3.5 are included in the PP Organic Inventory.

c. As defined in paragraph 4.3.6.

d. As defined in paragraph 4.3.7.

4.4 Robotic Spacecraft Assembly, Test, Transport, Launch, and Operations

4.4.1 Robotic planetary missions (lander, probe, orbiter, flyby, gravity assist) are to demonstrate that the management of bioburden during assembly, test, transport, and launch operations is sufficient to reach NASA cleanliness requirements, as outlined in Table 4-3. Lunar Category II, IIa, and IIb missions are excluded from bioburden management requirements.

Note: The Viking Program identified aerobic, mesophilic, heterotrophic spore forming organisms as good indicators of spacecraft cleanliness, as described in NHB 5340.1B, Microbiological Examination of Space Hardware.

4.4.2 Category II missions shall be built in a controlled manufacturing environment for which there are established and documented protocols for environmental, personnel, and hardware cleanliness in the PP Implementation Plan. Category II missions to Earth's Moon are excluded from this requirement.

Note: Assembly in a certified ISO Class 8 cleanroom in accordance with ISO 14644-1:2015 is an acceptable approach to achieve compliance with this

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requirement; however, Category II missions may propose alternate approaches to achieve compliance that meet the same intent.

4.4.3 Category III & IV missions shall be built in a minimum of an ISO Class 8 cleanroom in accordance with ISO 14644-1:2015(E).

4.4.4 Category III & IV missions shall develop an approach to demonstrate management of contamination in the PP Implementation Plan. Missions may achieve compliance using any single or a combination of methodologies provided in Appendix C. Acceptable methodologies include, but are not limited to, analysis of:

- a. A Bioburden Control Approach that may include:
 - (1) Bioburden at launch by addressing the following:
 - (a) Managing facility cleanliness.
 - (b) Managing bioburden.
 - (c) Accounting bioburden using:
 - (i) An agreed upon culture-based assay (Refer to Chapter 6).
 - (ii) Specification values for hardware biological cleanliness.
 - (iii) Alternative or augmented approaches (e.g., metagenomics analysis).
 - (d) Preventing recontamination.
 - (2) Breakup and burnup analysis of hardware.
- b. An Analytical Approach using the following parameters:
 - (1) Relevant elements of the Bioburden Control Approach, and
 - (2) Probability of encountering and impacting the target, including spacecraft reliability.
 - (3) Post-launch bioburden reduction based on environmental conditions experienced in deep space and on the surface of the target body.
 - (4) Mechanisms and timescales of transport to habitable environments at the target body, including surface impact location, lateral transfer, and the subsurface.

Note 1: For 4.4.4.a and 4.4.4.b above the alternative and analytical approaches are considered by the OPP on a case-by-case basis and reviewed by a non-advocate review board who submits findings and recommendations to the OPP for concurrence.

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Note 2: Missions can choose to propose genomic or metagenomics analysis as an alternative to culture-based bioburden accounting.

4.4.5 Category II, III, & IV missions shall document processes for notification of discoveries and anomaly events relevant to planetary protection during mission operations in the PP Implementation Plan.

4.4.6 Category III & IV missions shall document data and analyses used to demonstrate compliance with contamination avoidance requirements in the Pre-Launch PP Report.

4.4.7 Category III & IV missions that provide bioburden levels of the spacecraft and launch vehicle to demonstrate compliance with contamination avoidance requirements shall include a record of bioburden accounting data in the Pre-Launch PP Report.

4.4.8 Considerations that may impact planetary protection compliance (provided in Appendix D) occurring between Safety and Mission Success Review (SMSR) and Launch Readiness Review (LRR)/Flight Readiness Review (FRR) shall be communicated, in timely fashion, to feed into the decision-making process at LRR/FRR.

4.4.9 Updates to data and analyses provided per paragraphs 4.4.6 and 4.4.7 and any issues that arise after the delivery of the Pre-Launch PP Report, including considerations per paragraph 4.4.8, shall be documented in the Post-Launch PP Report.

4.5 Avoiding Contamination following Inadvertent Impact

4.5.1 Planetary missions (orbiter, flyby, gravity assist) are to demonstrate avoidance of contamination of sensitive solar system bodies by spacecraft and launched elements during flyby, gravity assist, orbital insertion, or orbital operations using current, scientific consensus approved planetary models.

Note: For missions conducting trajectories and maneuvers in the vicinity of sensitive solar system bodies (e.g., fly-by, gravity assist, or orbital), avoidance of inadvertent impact serves as a surrogate to demonstrating avoidance of contamination of potentially habitable environments.

4.5.2 Category II, III, & IV missions shall meet avoidance of contamination requirements by conducting an analysis of the probability of impact during mission planning, and if necessary, an analysis of probability of contamination following impact.

4.5.2.1 Category II, III, & IV missions shall design their trajectory and maneuvers based the appropriate probability of avoidance (see Section 4.5.3 through 4.5.6) of inadvertent impact at sensitive solar system bodies.

4.5.2.2 The applicable constraints to demonstrate avoidance of contamination due to inadvertent impact of sensitive solar system bodies are detailed in Sections 4.5.33 through 4.5.6.

4.5.3 Avoiding Contamination due to Inadvertent Impact for Category II Missions

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Category II missions to Jovian or Saturnian systems shall demonstrate contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) at sensitive solar system bodies in those systems (e.g., Europa and Enceladus) for 1,000 years.

4.5.4 Avoiding Contamination due to Inadvertent Impact of Mars

4.5.4.1 Category III and IV missions with launch vehicles leaving Earth orbit and cruise stages shall demonstrate contamination avoidance of Mars through one of the following approaches:

- a. Probability of impacting Mars is less than 1.0×10^{-4} for 50 years after launch.
- b. Total bioburden (surface, mated, and encapsulated) level is less than 5.0×10^5 spores at launch.

4.5.4.2 Category III and IV missions conducting trajectories and maneuvers in the vicinity of Mars (e.g., fly-by, gravity assist, or orbital) or launches from the surface of Mars shall demonstrate contamination avoidance of Mars through one of the following approaches:

- a. Probability of impacting Mars meets all of the following:
 - (1) Less than 1.0×10^{-2} for 20 years after launch.
 - (2) Less than 5.0×10^{-2} for a period extending from 20 to 50 years after launch.
- b. Total (surface, mated, and encapsulated) bioburden level is less than 5.0×10^5 spores at one of the following:
 - (1) Launch, as demonstrated by bioburden accounting.
 - (2) Planned impact at the Martian surface, as demonstrated by bioburden accounting followed by an approved breakup and burnup analysis of the hardware in the Martian atmosphere.

4.5.5 Avoiding Contamination due to Inadvertent Impact of Sensitive Icy Worlds

Category III missions conducting a fly-by or gravity assist of Europa, Enceladus, or other sensitive icy worlds to be determined shall demonstrate contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) for 1,000 years.

4.5.6 Avoiding Contamination due to Inadvertent Impact for Secondary and Auxiliary Payloads

4.5.6.1 Secondary and auxiliary payloads are subjected to inadvertent impact avoidance based on the detailed trajectory of the payload including flybys or gravity assists of solar system bodies objects. Typically, the primary mission conducts the inadvertent impact

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assessment of the launch vehicle but this should be confirmed by the secondary or auxiliary payload.

4.5.6.2 Secondary and Auxiliary Payloads Targeting a Solar System Body with More Stringent Implementation Requirements than Primary Payload

4.5.6.2.1 Category II and III secondary or auxiliary payloads targeting solar system bodies with more stringent implementation requirements than their primary payload shall avoid contamination due to inadvertent impact as per Table 4-3.

4.5.6.2.2 Category III secondary and auxiliary payloads targeting solar system bodies with more stringent implementation requirements than their primary payload shall prevent recontamination from the primary payload and launch vehicle elements.

4.5.6.3 Secondary and Auxiliary Payloads Whose Primary Payload is Targeting a Solar System Body with More Stringent Implementation Requirements

Category II and III secondary or auxiliary payloads whose primary payload is targeting a solar system body with more stringent implementation requirements shall implement a strategy for prevention and mitigation of recontamination of the primary payload.

Note: Such rideshare approaches may result in a need to conduct analyses to evaluate biological contamination between payloads in the pre-launch processing, launch or pre-separation phases that may require additional bioburden reduction or recontamination prevention for the secondary or auxiliary payload.

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Table 4-3. Avoidance of Contamination due to Inadvertent Impact Summary

Mission Description	Probability Assessment		Value Less Than	Duration or Timing	
Category II missions to Jovian or Saturn systems	Contamination Avoidance (occurrence for a biological inoculation event into a potentially habitable aqueous environment)		1.0×10^{-4} Probability	1,000 Years ^a	
Category II-IV missions launch vehicles leaving earth orbit and cruise stages	Contamination Avoidance of Mars	Inadvertent Impact of Mars	1.0×10^{-4} Probability	50 Years after Launch ^a	
		OR			
Category III or IV missions conducting trajectories and maneuvers in the vicinity of Mars (e.g., fly-by, gravity assist, or orbital) or launches from the surface of Mars	Contamination Avoidance of Mars	Inadvertent Impact of Mars	1.0×10^{-2} Probability	20 Years after Launch ^a	
			AND		
		5.0×10^{-2} Probability	20 to 50 Years after Launch ^a		
		OR			
		Total Bioburden Level	5.0×10^5 Spores	At Launch	
		OR			
		At Impact at the Martian Surface			
Category III or IV missions conducting a fly-by or gravity assist of Europa, Enceladus, or other sensitive icy worlds to be determined	Contamination Avoidance (occurrence for a biological inoculation event into a potentially habitable aqueous environment)		1.0×10^{-4} Probability	1,000 Years ^a	

a. Current period of biological exploration as defined by COSPAR approved by the COSPAR Bureau on 17 June 2020.

4.6 Avoiding Contamination for Robotic Landed Missions

4.6.1 Robotic planetary missions are to prevent occurrence of a biological inoculation event into a potentially habitable environment during landing and surface operations, including both stationary and mobile activities. Mission operation requirements may be achieved through either a bioburden control approach or an analytical approach demonstrated through a probability of occurrence analysis, as specified below. This section doesn't apply to Category IIa or IIb missions to the Earth's moon.

4.6.2 Missions Landing on Mars or Accessing the Martian Subsurface

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4.6.2.1 Category IVa missions to Mars shall demonstrate avoidance of contamination of Mars through one of the following approaches:

- a. Bioburden control approach that demonstrates all of the bioburden levels and applicable strategies for preventing contamination defined in Table 4-4 have been achieved.
- b. Analytical approach that provides a rigorous analysis demonstrating contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable environment.

Table 4-4. Category IV Robotic Missions to Mars Bioburden Control Requirements

Category		IVa	IVb	IVc		
Robotic Mission Description			Life Detection OR Sample Return	Special Region Access through Mobility	Landing within a Special Region	Credible Off-Nominal Landing Could Cause Contamination
Bioburden for All Landed Hardware	Total Spores (surface, mated, and encapsulated)	5.0×10^5	5.0×10^5	5.0×10^5	5.0×10^5	2.0×10^5
	Surface Spores (total and density)	3.0×10^5	3.0×10^5	3.0×10^5	30	30
		300 per m ²	300 per m ²	300 per m ²	3.0×10^{-2} per m ²	3.0×10^{-2} per m ²
Relevant Elements ^a	Surface Spores (total and density)	--	30	30	--	--
		--	3.0×10^{-2} per m ²	3.0×10^{-2} per m ²	--	--
Strategy for Preventing and Mitigating Recontamination of Relevant Elements ^b		No	Yes	Yes	Yes	Yes
Strategy for Preventing Contamination of Mars during EDL		No	No	Yes	Yes	Yes

a. Relevant hardware elements based on a mission description are defined in paragraph 4.6.2.2.a and 4.6.2.3.a.

b. If unaccountable hardware surfaces (e.g., High-Efficiency Particulate Air (HEPA)-filtered volumes, external cameras, electronic circuit cards) would exceed the surface or total bioburden level thresholds, then projects provide an assessment of reliability for Entry, Descent, and Landing (EDL) in the PP Implementation Plan.

4.6.2.2 Subcategory IVb (life detection investigation or sample return) missions shall demonstrate avoidance of contamination of Mars and preserve the integrity of the life detection experiment for the relevant hardware elements involved in the acquisition, delivery, and analysis of samples used for life detection through one of the following approaches:

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- a. A bioburden control approach that demonstrates all of the bioburden levels in Table 4-4 together with applicable strategies for preventing recontamination. Relevant elements to be reduced (through intentional processing or environmental exposure) are those that affect or are directly involved in the acquisition, delivery, or analysis of samples used for life detection.
- b. A more stringent bioburden control approach than Table 4-4 may be driven by the nature and sensitivity of the particular life detection experiments together with applicable strategies for preventing recontamination.

Note: The nature and sensitivity of the life detection instrument determines if more stringent cleanliness levels are needed to avoid “false positives” (detecting life when none is present) or “false negatives” (failing to detect life when it is present).

- c. An analytical approach that provides a rigorous analysis demonstrating that the integrity of the life detection experiment has been preserved. The approach should be as rigorous as the bioburden approach in Table 4-4.

Note: If all landed hardware is cleaned to a surface bioburden level of less than 30 spores, then the requirement to prevent recontamination is met.

4.6.2.3 Subcategory IVc (special region access) missions for which the landing ellipse does not include special regions but plan to access a special region through horizontal or vertical mobility shall demonstrate avoidance of contamination of Mars through one of the following approaches:

- a. Bioburden control approach that demonstrates all of the bioburden levels and applicable strategies for preventing contamination defined in Table 4-4 have been achieved. Relevant elements to be reduced (through intentional processing or environmental exposure) are those that directly contact the special region.
- b. Analytical approach that provides a rigorous analysis demonstrating contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable environment.

4.6.2.4 Subcategory IVc (special region access) missions landing within a special region shall demonstrate avoidance of contamination of Mars through one of the following approaches:

- a. Bioburden control approach that demonstrates all of the bioburden levels and applicable strategies for preventing contamination defined in Table 4-4 have been achieved.
- b. Analytical approach that provides a rigorous analysis demonstrating contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable environment.

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4.6.2.5 Subcategory IVc (special region access) missions where a credible off-nominal condition could cause high probability of inadvertent biological contamination shall demonstrate avoidance of contamination of Mars through one of the following approaches:

- a. Bioburden control approach that demonstrates all of the bioburden levels and applicable strategies for preventing contamination defined in Table 4-4 have been achieved.
- b. Analytical approach that provides a rigorous analysis demonstrating contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable environment.

4.6.2.6 Category IV missions to Mars shall provide in the Pre-Launch PP Report a characterization of potential special region access in a nominal or credible off-nominal condition based on findings from the landing site selection process and related scientific data.

4.6.3 Missions Landing on Mars or Accessing a Sensitive Icy World's Subsurface

4.6.3.1 Category IV missions landing on or accessing the subsurface of Europa, Enceladus, or other sensitive icy worlds to be determined shall demonstrate contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) for 1,000 years.

4.6.3.2 Category IV life detection investigations or sample return missions landing on or accessing the subsurface of Europa, Enceladus, or other sensitive icy worlds to be determined shall determine the minimum cleanliness levels needed for the relevant hardware elements driven by the nature and sensitivity of the particular life detection experiments together with applicable strategies for preventing recontamination.

Note: The nature and sensitivity of the life detection instrument determines if more stringent cleanliness levels are needed to avoid "false positives" (detecting life when none is present) or "false negatives" (failing to detect life when it is present).

4.6.4 Avoiding Contamination for Secondary and Auxiliary Payload Lander Missions with More Stringent Implementation Requirements than Primary Payload

Category IV secondary and auxiliary payloads that are targeting solar system bodies with more stringent implementation requirements than their primary payload shall implement a strategy for prevention and mitigation of recontamination by the primary payload.

4.7 End of Mission Disposition

4.7.1 Category II, III, IV, and Unrestricted Earth Return V(u) missions shall document the final disposition of hardware elements, including any updates to organic, biological, and combustion products inventories, in the End of Mission PP Report.

Note: Category Restricted Earth Return V(r) missions are considered in Chapter 5.

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4.7.2 Category II orbiter and III robotic missions shall report any changes to the probability of inadvertent impact avoidance as a result of prior changes to mission operations, credible off-nominal spacecraft performance, accelerated spacecraft degradation, or anomaly events in the End of Mission PP Report.

5. PRECLUDE BACKWARD CONTAMINATION

5.1 Purpose

This chapter provides requirements to rigorously preclude backward contamination of the Earth-Moon system by extraterrestrial life or bioactive molecules in returned samples from habitable solar system bodies. This is accomplished by preventing organisms present at the destination from being introduced into Earth’s biosphere. Acceptable methodologies include prescriptive or performance based (e.g., assurance case) approaches. See Figure 5-1 for an overview of preventing backward contamination.

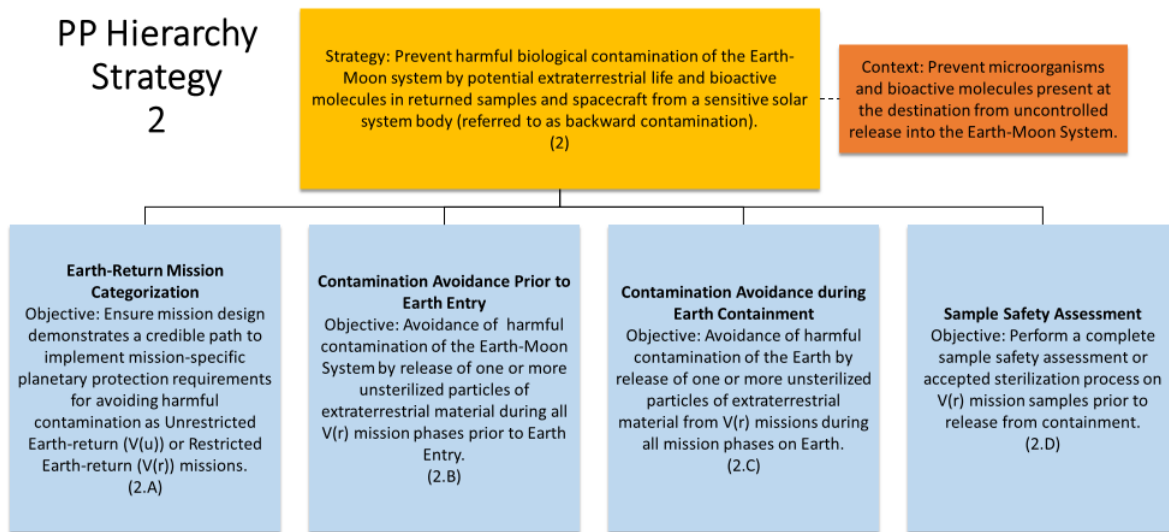


Figure 5-1. PP Strategy 2 – Prevent Backward Contamination

5.1.1 The entirety of the chapter is applicable to robotic missions. Currently, only unrestricted Earth-Return mission V(u) categorization are applicable to crewed missions (i.e., bringing samples back from the Moon and e.g., near Earth asteroids). Crewed mission implementations for restricted Earth-Return V(r) will be updated as knowledge gaps are closed and policy objectives are finalized.

5.2 Categorization Elements for Robotic and Crewed Earth-Return Missions

5.2.1 Earth-return missions shall propose an inbound category using information in NPR 8715.24 as the basis for the proposed categorization (further characterized in Appendix A). The assignment of missions to an Earth-return categorization is based on the following:

- a. For missions to target bodies not assigned an Earth-return categorization in NPR 8715.24, justification for unrestricted Earth-return (V(u)) is based on the scientific evidence that the mission will not cause harmful biological contamination of the Earth’s biosphere. See paragraph A.1.3 for additional information.

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b. If the scientific evidence does not support the Category V(u) categorization or if scientific evidence is not provided, then the mission will be assigned Category V(r).

5.2.2 Robotic restricted Earth-return (V(r)) missions shall provide a notional timeline for the development and commissioning of a sample receiving facility (SRF) to contain the sample and conduct a sample safety assessment. The timeline should include instrumentation and operations as part of the planetary protection categorization proposal because of the lengthy time needed for the complex development of an SRF and its associated biohazard-test.

5.2.3 Unrestricted Earth-return (V(u)) missions do not require further planetary protection considerations for the return phase of the mission.

5.3 Robotic Restricted Earth-Return (V(r)) Reporting Requirements

5.3.1 Category V(r) missions shall document the actual schedule for reports and reviews necessary to demonstrate PP compliance to support decision making processes for Earth-Return, Earth-Entry and sample release from containment in the PP Requirements Document.

Note: These reports and reviews align with project life cycle critical event readiness reviews (CERRs) or events in mission operations.

5.3.2 Category V(r) missions shall develop an approach to demonstrate avoidance of contamination of the Earth-Moon System in the PP Implementation Plan.

5.3.3 Considerations that may impact planetary protection compliance occurring between Post-Launch Assessment Review (PLAR) and Earth-Return preparations shall be communicated, in timely fashion, to feed into the decision-making process for returning samples back to Earth.

5.3.4 Category V(r) mission shall document data and analyses used to demonstrate compliance with contamination avoidance requirements prior to samples returning to Earth.

5.3.5 Considerations that may impact planetary protection compliance occurring between Earth-Return and Earth-Entry shall be communicated, in timely fashion, to feed into the decision-making process at Earth-Entry.

5.3.6 Updates to data and analyses provided per paragraph 5.3.3, confirmation of a containment facility readiness, shall be documented prior to samples returning to Earth.

5.3.7 Programs managing restricted samples shall develop an approach to demonstrate avoidance of contamination of the Earth in the Sample Pre-Release Report, to feed into the decision-making process for releasing samples out of containment..

5.4 Restricted Earth-Return (V(r)) Implementation Requirements

Note: The highest level of biological concern is to be assumed for unsterilized extraterrestrial material until the samples are either analyzed for extraterrestrial life and bioactive molecules or sterilized and/or inactivated.

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5.4.2 Category V(r) missions shall demonstrate avoidance of harmful contamination of the Earth-Moon System by release of one or more unsterilized particles into the Earth-Moon system of extraterrestrial material during all mission phases prior to Earth entry leveraging one or more of the following approaches, or a combination of them:

Note: An unsterilized particle size of concern is 10 nm or greater in demonstrating contamination avoidance of the Earth or the Moon.

a. An assessment that leverages strategies and design concepts that assure containment of target body-exposed Earth-return hardware factoring in the following:

Note: Strategy and design concepts are considered on a case-by-case basis and reviewed by a non-advocate review board who submits findings and recommendations to the OPP for concurrence.

Note: Missions may achieve avoidance of harmful contamination of the Earth-Moon System by demonstrating the probability of releasing one or more unsterilized particles of extraterrestrial material is less than 1.0×10^{-6} for each phase.

(a) Likelihood of the presence of uncontained, unsterilized V(r) target material on the returning spacecraft hardware.

Note: For example, through the assessment of approaches to "break the chain of contact" with the target.

(b) Fidelity of mission design and diversion operations, including accuracy of delivery and impact avoidance at the Earth-Moon System of target-exposed spacecraft hardware within 100 years of initiation of Earth-return trajectory.

(c) Containment performance of the entry vehicle in the case of a nominal landing or capture.

(d) Potential for a credible off-nominal landing on the Earth or Moon surface or a failed in-space capture activity.

b. Sterilization and inactivation of samples prior to entry into the Earth-Moon System using a process that focuses on terrestrial degradation mechanisms occurring at a molecular level sufficient to inactivate terrestrial bioactive molecules present with a high degree of assurance.

Note: This assumes that extraterrestrial biological systems would be susceptible to the same inactivation mechanisms as terrestrial biological systems from a biochemical and biophysical perspective.

Note: Sterilization and inactivation of samples may be achieved by leveraging overkill levels of terrestrial sterilization or inactivation of bioactive molecules through implementation of sterilization industry standards (e.g., ISO 11138-1:2017, ISO 14937:2009, ISO 22442 3:2007).

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c. Ensuring contaminated hardware or samples does not enter the Earth-Moon System.

5.4.3 Category V(r) missions shall demonstrate avoidance of harmful contamination of the Earth by release of one or more unsterilized particles of extraterrestrial material during all mission phases on Earth leveraging one or more of the following approaches:

a. Category V(r) missions shall demonstrate avoidance of harmful contamination of the Earth by release of unsterilized extraterrestrial material through containment upon Earth entry in the following phases:

(1) At the landing site.

(2) During transport.

(3) During storage, curation and sample safety assessment activities.

Note: A biocontainment facility in accordance with biosafety best practices and the highest level of biocontainment (e.g., Centers for Disease Control and Prevention (CDC)/ National Institutes of Health (NIH) June 2020) may be an acceptable means to contain spacecraft hardware and samples returned from restricted target bodies.

b. Extraterrestrial samples returned as part of a restricted Earth-return V(r) mission shall undergo a sample safety assessment demonstrating the sample is not harmful prior to unrestricted sample release.

Note: Sample safety assessment shall be performed based on current scientific consensus addressing sensitivity, specificity, sub-sampling approach, test-sequence, decision criteria as described in Kminek et al. 2022.

Note: The current sample safety assessment framework has been studied with the context of Mars return samples. Other target bodies may require further assessment.

c. Extraterrestrial sample sterilization shall use a process that focusing on terrestrial degradation mechanisms occurring at a molecular level sufficient to inactivate terrestrial bioactive molecules present with a high degree of assurance prior to unrestricted sample release.

Note: Sterilization and inactivation of samples may be achieved by leveraging overkill levels of terrestrial sterilization or inactivation of bioactive molecules through implementation of sterilization industry standards (e.g., ISO 11138-1:2017, ISO 11138-7:2019-3, ISO 14937:2009, ISO 22442-3:2007).

Note: Sterilization processes may be verified by using an analog organism that is known to be resistant to the chosen sterilization modality in an analog geological matrix that is similar to the sample being sterilized or inactivated.

5.4.4 Missions conducting life detection investigations or sample safety assessments shall include strategies to avoid “false positives” and “false negatives” signals.

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Note: False positives may prevent distribution of the sample from containment and could lead to unnecessary increased rigor in the requirements for all subsequent sample return missions.

6. NASA STANDARD SPORE ASSAY

6.1 Method Overview and Applicability

6.1.1 This chapter provides requirements for the NASA accepted approach to performing spore assays for accounting bioburden as part of the bioburden control approach to demonstrate compliance with forward contamination avoidance requirements.

6.1.2 The method to be used for accounting bioburden shall either be the procedure outlined below in section 6.2 – 6.8 or ECSS-Q-ST-70-55C Microbial examination of flight hardware and cleanrooms (15 November 2008).

6.2 Introduction

6.2.1 All operations involving the manipulation of sterile items and sample processing shall be performed in an aseptic environment.

Note: For all analyses, the assay procedures to be performed, the number of assays, the duration of exposure, and the number of sites analyzed is considered by the OPP on a case-by-case basis and reviewed by a non-advocate review board who submits findings and recommendations to the OPP.

6.3 Preparation

6.3.1 Preparation of Media and Reagents

6.3.1.1 The water used in all analyses for the NASA standard spore assay processes shall be ASTM type II in accordance with ASTM D1193-06(2018).

6.3.1.2 Tryptic Soy Agar (TSA) powder shall be prepared and sterilized in accordance with the manufacturer instructions.

6.3.1.3 Spore standard used in all analyses shall be *Bacillus atrophaeus* ATCC 9372 (DSM 675), hereafter referred to as spore standard.

6.3.1.4 Stock buffer solution shall be prepared in the following method:

- a. Dissolve 34.0 g of potassium dihydrogen phosphate (KH₂PO₄) in 500 mL of distilled water.
- b. Adjust to pH 7.2±0.1 with 1N NaOH and dilute to one liter with distilled water.

6.3.1.5 Buffered distilled water shall be prepared by adding 1.25 mL of the stock buffer solution to 1 L of sterile, distilled water.

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6.3.1.6 Sterile rinse solution shall be prepared as follows:

- a. Add a 0.02 percent volume-to-volume solution Tween 80 in buffered distilled water to reach a final pH of 7.1-7.3.
- b. Sterilize the resulting solution by autoclaving in accordance with section 6.3.2.

6.3.2 Preparation of Sterile Goods

Utilization of an autoclave in performing NASA standard spore assay shall include all of the following:

- a. For autoclaving dry or liquid materials, minimum sterilization conditions are 121°C at 1.03 bar (15 psi) for 15 minutes.
- b. Autoclave performance is validated with biological indicator organisms or other standard validation methods consistent with industry standards (e.g. ISO 11138-1:2017).
- c. Laboratory supplies (e.g., forceps, test tubes, petri plates) are to be sterilized or purchased sterile.

6.3.3 Selection and Preparation of Swabs

Swabs utilized in NASA standard spore assay shall be:

- a. Commercially available absorbent swabs firmly twisted to a suggested size of 5 x 19 mm long over one end of an applicator stick.
- b. Sterilized and packaged in a protective container.

6.3.4 Selection and Preparation of Wipes

Wipes utilized in NASA standard spore assay shall be:

- a. Commercially available 100 percent polyester-bonded cleanroom wipes, 23 x 23 cm.
- b. Dampened with 15 -18 mL of water.
- c. Sterilized and packaged in an individual container.

6.4 Instrumentation

6.4.1 Sonicator

All sonication procedures shall conform to the following specifications:

- a. Inside surfaces of the bath made of stainless steel.

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- b. Bath fluid consisting of an aqueous solution of 0.02 percent volume-to-volume of polyoxyethylene sorbitan monooleate to water.
- c. Make up the aqueous solution fresh each day.
- d. The temperature of the bath fluid is to be:
 - (1) Greater than or equal to 20°C.
 - (2) Less than or equal to 32°C.
- e. Run the bath for at least 5 minutes prior to sonicating samples to be assayed to de-gas the bath.

6.4.2 Pilot container

Containers identical to those used for the assay samples shall be used as a pilot container following the process defined below:

- a. Fill one pilot container with the same volume of solution used for the test samples.
- b. Place a calibrated measuring device in the pilot container to monitor:

When the temperature of the container reaches the required temperature.

Record temperature during heat shock.

6.5 Sampling

6.5.1 Throughout the sampling process, aseptic techniques shall be used. This includes, but is not limited to, the use of sterile gloves, sterile forceps, sterile wipes, sterile swabs.

6.5.2 Swab Sampling

6.5.2.1 The following protocol shall be used for swab sample collection.

- a. Limit sample collection from surfaces less than or equal to 25 cm² per swab.
- b. Use sterile test tubes containing 10 mL of sterile distilled water for each sample. Prepare enough tubes to accommodate all samples to be collected plus controls.
- c. Remove a sterile cotton swab from its container and moisten the head of the swab in a test tube.
- d. Remove excess moisture from the swab against the interior wall of the tube.
- e. Aseptically collect the sample by rotating the head of the swab while moving in one direction across the surface, and repeat on the area of interest horizontally, vertically, and diagonally.

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f. Return the head of the swab to the original tube containing sterile distilled water, breaking off the head of the swab against the side of the tube below any portion of the handle that was touched by the sampler.

g. A negative control shall be collected for every ten or fewer samples.

h. Initiate sample processing within 1 hour; otherwise, the samples may be stored for up to 24 hours at 4°C.

6.5.3 Wipe Sampling

6.5.3.1 The following protocol shall be used for wipe sample collection:

a. Collect a sample over a surface area between 0.1 and 1.0 m².

b. Use between 35% and 75% of the area of the wipe used for sampling.

c. Use an aseptic technique for sample collection.

d. A negative control shall be collected for every six or fewer samples.

e. Initiate sample processing within 1 hour; otherwise, the samples may be stored for up to 24 hours at 4°C.

6.6 Sample Processing

6.6.1 Throughout all these assays, aseptic techniques shall be used. This includes, but is not limited to, the use of sterile gloves, sterile forceps, sterile wipes, sterile swabs.

6.6.2 Swab Processing

6.6.2.1 A positive control standard shall be prepared at the desired spore concentration and carried through all subsequent steps.

6.6.2.2 A negative control containing an unused swab shall be prepared and carried throughout all subsequent steps.

6.6.2.3 Extraction

6.6.2.3.1 Each sample shall be extracted using the following procedure:

a. Vortex each sample thoroughly for 5 to 6 seconds.

b. Sonicate each sample for 2 minutes ± 5 seconds.

Note: Elevate the bath fluid above the level of the liquid in the flasks being sonicated.

Note: Free the flasks and tubes of contact with other surfaces and fully suspend in the bath.

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6.6.2.3.2 Each sample shall be heat shocked as follows:

- a. Place the sample containing the vortexed solution and the swab head in a water bath at 78-82°C for 15 minutes, as determined by a pilot container containing a calibrated measuring device in 10 mL of water.
- b. Cool the samples immediately in an ice bath, held for no longer than 45 minutes prior to plating.

6.6.2.4 Each sample shall be plated by aseptically pipetting the extraction fluid into Petri plates, using 8.0 mL of total fluid.

6.6.2.5 Approximately 20 mL of sterile, molten (48-50°C) TSA shall be added to each plate; mix the contents by gently swirling; and allow the mixture to solidify at room temperature.

6.6.2.6 A least one water-only blank shall be plated as a negative control.

Note: It is recommended to pour TSA blanks during plating.

6.6.3 Wipe Processing

6.6.3.1 A positive control standard shall be prepared at the desired spore concentration. The control standard is carried through all subsequent steps.

6.6.3.2 A negative control containing an unused wipe shall be prepared and carried throughout all subsequent steps.

6.6.3.3 Extraction

6.6.3.3.1 Each sample shall be extracted using the following procedure:

- a. Add 200 mL of rinse solution to each individual sample.
- b. Vortex each sample thoroughly for 5 to 10 seconds.
- c. Sonicate each sample for 2 minutes \pm 5 seconds.

Note: Elevate the bath fluid above the level of the liquid in the flasks being sonicated.

Note: Free the flasks and tubes of contact with other surfaces and fully suspended in the bath.

6.6.3.3.2 Each sample shall be heat shocked as follows:

- a. Place the sample containing the vortexed solution and the wipe in a water bath at 78-82°C for 15 minutes, as determined by a pilot container with a calibrated measuring device in 200 mL of water.

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- a. Cool the samples immediately in an ice bath, held for no longer than 45 minutes prior to plating.
- b. Each sample needs to be plated by aseptically pipetting the extraction fluid into Petri plates, using a total of 50 ml fluid.

6.6.3.4 Approximately 20 mL of sterile, molten (48-50°C) TSA shall be added to each plate; mix the contents by gently swirling; and allow the mixture to solidify at room temperature.

6.6.3.5 A least one rinse blank shall be plated as a negative control.

Note: It is recommended to pour TSA blanks during plating for each batch of TSA.

6.7 Counting

6.7.1 The following procedure shall be followed to obtain spore counts as a result of conducting the NASA standard spore assay:

- a. Incubate all samples, controls, and blanks aerobically at 32°C in an inverted position.
- b. Perform the final count at the 72-hour interval.
- c. If colonies are observed, count and record data at 72 hours.

Note: Additional counting is recommended at 24 and 48 hours to monitor for any potential contamination.

- d. Express results as a total number of microorganisms per unit area when a surface is being assayed.

6.7.2 Data from 6.7.1 shall be analyzed using a recognized statistical approach to obtain spacecraft bioburden estimates.

6.8 Quality Assurance and Quality Control

6.8.1 Corrective action measures shall be implemented if quality assurance and quality control requirements are not met.

6.8.2 Sterility and contamination controls shall be processed as part of each assay.

6.8.2.1 Positive Controls.

6.8.2.1.1 A spore solution containing spore standard shall be plated for each assay.

6.8.2.1.2 Spore standard shall be assessed by plating out appropriate controls for each assay.

6.8.2.2 Negative Controls.

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6.8.2.2.1 An unused swab or wipe shall be processed for each sampling event when the device is used.

6.8.2.3 Each batch of TSA shall be tested for sterility.

6.8.2.4 Accuracy and Reproducibility

6.8.2.4.1 Accuracy and reproducibility of the procedures shall be internally validated on an annual basis.

6.8.2.4.2 Records documenting the calibration of select instrumentation used for the accuracy and reproducibility of the methods used shall be maintained by any laboratory performing NASA standard spore assay.

6.8.2.4.3 Records documenting the accuracy and reproducibility of the methods used shall be made available to NASA management upon request.

APPENDIX A. PP MISSION CATEGORIZATION PROPOSAL—EXPECTED ELEMENTS

A.1 Overview

A.1.1 The project's PP Mission Categorization Proposal must include the required elements provided in sections 4.2 and 5.2. The preliminary proposal is delivered as per the deliverable timeline defined in NPR 8715.24. The following expected elements are provided as part of the as substantiation for the project's proposed planetary protection category.

Note: Analysis of the following elements is expected to be at a level of fidelity and detail needed to make a planetary protection category determination.

For missions to targets with undetermined categorizations consult with the PPO as per NPR 8175.24 Section 3.1.

A.1.2 The mission description provided as part of the PP Mission Categorization Proposal includes an impact probability analysis under the proposed spacecraft trajectory if robotic planetary missions and missions making close approaches to Mars, Europa, and Enceladus.

A.1.2.1 A deterministic analysis is appropriate for missions not targeting Mars, Europa, or Enceladus.

A.1.2.2 Analyses at Mars are based on spacecraft trajectory, while analyses at Europa and Enceladus involve spacecraft reliability.

A.1.3 Missions targeting a sensitive solar system body describe the approach to meeting the cleanliness requirements, addressing any specific cleanliness interfaces between the primary and secondary payloads. Alternative approaches are permitted following review and concurrence by the relevant authorities.

A.1.3.1 Projects provide relevant scientific knowledge of the target body, including the current understanding of relevant geological features, icy and aqueous environments, and the prospects of accessing them.

A.1.3.2 Missions encountering multiple solar system bodies need to address every solar system body along their trajectory to obtain the appropriate categorization. The driving planetary protection category would be based on the most stringent solar system body category encountered.

A.1.4 Category IV missions to Mars should provide the specific cases for special region access, which include:

a. Landing site within a potential special region (e.g. gullies, confirmed and partially confirmed Recurrent Slope Lineae (RSL), groundwater, sources of methane, geothermal activity, modern outflow channel, others on a case-by-case basis) in the upper 5m of the subsurface.

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- b. Operational access through horizontal or vertical mobility (e.g. gullies, confirmed and partially confirmed RSL, groundwater, sources of methane, geothermal activity, modern outflow channel, subsurface cavities, caves, subsurface below 5m, others on a case-by-case basis).
- c. Identification of a spacecraft induced special region (e.g. nuclear reactor heat, radioisotope heat or power source, spacecraft commodities or payload that would increase water availability).
- d. Evaluation of the special region must be based on the latest scientific evidence to include an assessment of the extent to which the temperature and water activity values specified for Mars Special Regions are separated in time.

A.1.4.1 A Special Region is defined as a region within which terrestrial organisms are likely to replicate. Any region which is interpreted to have a high potential for the existence of extant Martian life forms is also defined as a Special Region.

A.1.4.2 Given current understanding of terrestrial organisms, Special Regions are defined as areas or volumes within which sufficient water activity and sufficiently warm temperatures to permit replication of Earth organisms. The physical parameters delineating applicable water activity and temperature thresholds are given below based on Rummel et al., 2014:

- a. Lower limit for water activity: 0.5; upper limit: 1.0.
- b. Lower limit for temperature: -28°C; no upper limit defined.
- c. Timescale over which limits apply: 500 years.

A.1.4.3 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 are further defined on that basis (Kminek et al., 2010, Rummel et al., 2014).

A.1.5 Justification for unrestricted sample return is typically based on the elements described in “Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies”, provided below:

- a. Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?
- b. Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?
- c. Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO₂ or carbonates and an appropriate source of reducing equivalents) in or on the target body to support life?

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- d. Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., $>160^{\circ}\text{C}$)?
- e. Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life forms?
- f. Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

APPENDIX B. PP REQUIREMENTS DOCUMENT—EXPECTED ELEMENTS

B.1 Purpose

B.1.1 The PP Requirements Document specifies the project’s planetary protection project-level requirements, including the identified standards the project intends to leverage to achieve compliance. The requirements document is delivered as per the deliverable timeline defined in NPR 8715.24.

Note: Analysis of the following elements is expected to be at a level of fidelity and detail needed to demonstrate compliance.

B.2 Planetary Protection Requirement Approaches

B.2.1 The following are the expected elements that are to be included as part of the PP Requirements Document:

- a. Identified applicable Agency planetary protection requirements from NPR 8715.24 and NASA-STD-8719.27 that are consistent with the planetary protection categorization covering the project life-cycle.
- b. Agreed upon set of accepted standards to achieve compliance with the identified applicable project-level planetary protection requirements (see paragraph B.2.2 below).

Note: Other standards may be applicable and are to be identified by the mission and concurred by the OPP. The list of standards provided below are standards that have been reviewed by OPP and determined to meet compliance.

- c. Mission’s are to define their approach and compliance status to the identified applicable project-level planetary protection requirements, as specified below:

- (1) Compliant.
- (2) Not Applicable.
- (3) Non-Compliant

Note: Known non-compliances are addressed with appropriate mitigations provided and agreed to by the OPP ahead of the review of the PP Requirements Document at System Requirements Review (SRR).

- d. Established schedule for delivery of the documents required to demonstrate compliance with planetary protection requirements in coordination with the OPP as per NPR 8715.24.

Note: Restricted Earth-Return mission may require additional sample return report(s) prior to Earth return, Earth entry, and sample release.

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Note: The OPP works with each mission for the time needed to review document prior to life-cycle reviews.

APPENDIX C. PP IMPLEMENTATION PLAN—EXPECTED ELEMENTS

C.1 Purpose

C.1.1 The PP Implementation Plan specifies the project’s planetary protection implementation activities covering the project life-cycle to achieve compliance with the established project-level planetary protection requirements documented in the Planetary Protection Requirements Document, including alternate approaches to achieve compliance.

C.1.2 The following annotated outline provides the kind of information that is expected to be described within the PP Implementation Plan. The amount and depth of description will vary based on the PP mission categorization.

Note 1: For Category II missions only a brief plan outlining the intended or potential impact targets is required. Additional inputs such as the management and facility information is not required.

Note 2: For Category III missions, if the mission involves an orbiter, ensure that the minimum planned periapsis altitude and planned final disposition of the hardware is to be documented.

C.2 Planetary Protection Implementation Plan Outline

C.2.1 Project Organization and Responsibility

C.2.2 Project Integrated PP Reporting Schedule

C.2.3 Mission Description

C.2.4 Planetary Protection Requirements

C.2.5 PP Compliance Risk Assessment

C.2.6 Approach to Compliance – performance or assurance case-based approach

C.2.7 Implementation

C.2.8 Bioburden Control

C.2.9 Supporting Analysis

C.2.10 Reviews and Additional Deliverables

C.3 Planetary Protection Implementation Approaches

C.3.1 Category III & IV missions develop an approach to demonstrate avoidance of contamination. Acceptable methodologies include:

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C.3.1.1 A Bioburden Control Approach that may include:

a. Bioburden at launch by addressing:

(1) Managing facility cleanliness.

Accepted Standard: (e.g., ISO 14644 Series provided in Chapter 2 – AAMI TIR52:2014/(R)2017, ECSS-Q-ST-70-55C 15 November 2008)

- (a) Personnel gowning protocols (ISO 14644 5:2005).
- (b) Ingress and egress control of essential and non-essential personnel, occupancy level, materials, equipment, and hardware into/out of the facility.
- (c) Control over materials stored in the room.
- (d) Control of contaminant-generating processes in the room (e.g. soldering, filing, painting, etc.).
- (e) Facility maintenance (e.g. cleaning schedule, processes, materials, and solvents to be used). Cleanroom qualification plan and certification frequency. Monitoring plan for particulates and humidity.
- (f) Baseline monitoring of biodiversity on surfaces in the assembly facility prior, during, and following hardware assembly. Environmental monitoring program alert and action levels.
- (g) A framework for anomaly recognition and mitigation strategies for external impacts to cleanroom operations during planned and unplanned interruptions (e.g. environmental incidents such as wildfires, hurricanes, tornados and human induced incidents such as landscaping maintenance, pest control).
- (h) Identification of the facility manager responsible for maintenance, access, and correction of issues.

(2) Managing bioburden.

Accepted Standard: (e.g., ECSS-Q-ST-70-55C 15 November 2008)

- (a) Bioburden allocation for mission and subsystem level hardware.
- (b) Bioburden accounting including surface, mated, and encapsulated surfaces of flight hardware at identified points in assembly and integration (e.g., inspection, last-access, and delivery events).

b. Implementation of bioburden assays to quantitate surface density and calculate total bioburden load of heat resistant spores (see ECSS-Q-ST-70-57C 30 August 2013).

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- (a) Bioburden reduction using verified methods or post reduction bioassay as appropriate for spacecraft materials (see ECSS-U-ST-20C 1 August 2019).
- (b) An approach to identify and archive culture isolates from flight hardware bioassays for long term storage.
- (c) Recontamination prevention through use of cleanroom assembly, ground support equipment, and flight biobarriers.
- (d) A framework for anomaly recognition and mitigation strategies to recover from biological contamination.
- (e) An approach to the use of bioinformatics for assessing diversity and relevance of bioburden in facilities and on spacecraft.
- (f) Maintain all hardware to visibly clean levels before moving on to next step in assembly, integration, test, and transport process (see IEST-STD-CC1246E).

Note: Acceptable approaches to demonstrate bioburden control include classical culturing or genomics-based assessment (including enumeration, phylogenetic identification, and high-resolution characterization of microbial traits and biochemical capabilities) of microorganisms on spacecraft. Bioburden control approach needs to include assessment of remaining viable organisms or sterility assurance level.

(3) Accounting bioburden using:

Accepted Standard: (e.g., AIAA S-120A-2015(2019))

- (a) Using the maximum value in Table C-2 encapsulated and enclosed surface bioburdens, unless testing has been done to justify accounting at a lower value.
- (b) Tracing manufacturing provenance (including raw materials origin, processes, and treatments), providing a rationale for selection of values less than the maximum of the applicable range.

Table C-1. Spore Surface Manufacturing Provenance Bioburden

Surface Density^A	
ISO 7 cleanroom or better, biologically controlled	50/m ²
ISO 7 cleanroom, particle control	500/m ²
ISO 8 cleanroom, biologically control	1×10 ³ /m ²
ISO 8 cleanroom, particle control	1×10 ⁴ /m ²

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Uncontrolled manufacturing	$1 \times 10^5 / \text{m}^2$
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^AFor estimating surface densities for vegetative microorganisms multiply values by a factor of 10.

(c) Providing a direct assessment using an approved approach of material as outlined.

(d) Account a lower bioburden based on application of an approved bioburden reduction process (e.g., Heat Microbial Reduction (HMR), Vapor Hydrogen Peroxide). Approved bioburden reduction processes include:

(i) Surface and encapsulated heat microbial reduction (ECSS-Q-ST-70-57C 30 August 2013)

(ii) Time-temperature for absolute sterility must reach a temperature of at least 500°C and remain at this temperature for at least 0.5 second.

(iii) Vapor hydrogen peroxide microbial reduction (ECSS-Q-ST-70-56C 30 August 2013)

(iv) Alternative processes can be proposed to include industrial sterilization standards or space environmental models.

(e) Agreed upon culture-based assay (refer to Chapter 6).

(f) Specification values for hardware biological cleanliness.

Table C-2. Encapsulated and Enclosed Surface Bioburdens

Encapsulated Organisms	
Electronic Piece Parts	$3-150 / \text{cm}^3$
Nonmetallic Material, Average	$1-30 / \text{cm}^3$
Other Nonmetallic Materials	$1-30 / \text{cm}^3$
Enclosed Surface Organism Densities	
Cleanroom – Particle and Biologically Controlled	$0.05-0.5 / \text{cm}^2$
Cleanroom – Only Particle Controlled	$0.5-10 / \text{cm}^2$
Uncontrolled Manufacturing	$10-100 / \text{cm}^2$

(g) Alternative or augmented approaches (e.g., metagenomics analysis).

(4) Preventing recontamination.

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Accepted Standard: (e.g., ECSS-Q-ST-70-01C 15 November 2008)

- (a) Provisions for packaging, containerization, transportation, and storage in order to maintain biological cleanliness of cleaned flight hardware coming into contact with other surfaces (e.g., GSE, purge equipment, test fixtures) following assembly up to launch, including the accommodation of witness plates.
- (b) Use of packaging materials and components that maintain the flight hardware biological cleanliness and prevent recontamination.
- (c) Prevention of shed particles and foreign object debris as both types of materials serve as potential vectors for biological contamination.
- (d) Evaluation and verification of hardware biological cleanliness prior to integration with packaging materials or components during the containerization, transport, or storage of the cleaned flight hardware.
- (e) Protocols for maintaining flight hardware cleanliness and avoiding wear debris during transportation, taking into account exposure to environmental fluctuations (e.g., temperature, pressure, and humidity) and vibrations.
- (f) Packaging materials and components used for hardware storage that are designed to adequately maintain flight hardware cleanliness for the planned length of storage duration.
- (g) Monitoring and periodic verification of packaging integrity and hardware that is maintained in storage for longer than planned to ensure hardware cleanliness levels are maintained.
- (h) Appropriate environmental controls to maintain the biological cleanliness for clean flight hardware.
 - (i) Record of final bioburden accounting data. Should use an accounting tool and make that available to the OPP for review. Deliver electronic final version of that to the OPP for concurrence.

c. Breakup and burnup analysis of hardware.

C.3.1.2 An Analytical Approach using the following parameters:

- a. Relevant elements of the Bioburden Control Approach, and
- b. Probability of encountering and impacting the target, including spacecraft reliability.
 - (5) Probability of encountering/landing on the target, including spacecraft reliability
 - (6) Probability of surviving landing/impact on the target

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c. Post-launch bioburden reduction on the basis of environmental conditions experience in deep space and on the surface of the target body.

(7) Bioburden of relevant viable organisms at launch

(8) Cruise survival for contaminating organisms

(9) Organism survival in the radiation environment adjacent to the target.

d. Mechanisms and timescales of transport to habitable environments at the target body, including surface impact location, lateral transfer, and the subsurface.

(10) Mechanisms and timescales of transport to the subsurface

(11) Organism survival and proliferation before, during, and after subsurface transfer

(12) Scale of contaminable environment.

Note: Typically, this is initially addressed through a probability of impact assessment (also at 1.0×10^{-4}), but other methodologies may be required if this approach is insufficient (e.g., ISO 11231:2019, NASA/SP-2011-3421, NASA/SP-2011-3422) from the items above.

C.3.2 Missions Landing on or Accessing a Sensitive Icy World's Subsurface

Category IV missions landing or accessing the subsurface on Europa, Enceladus, or other sensitive icy worlds demonstrate contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) for 1,000 years. Demonstrating contamination avoidance can be achieved through data and analyses of:

a. Bioburden at launch.

b. Cruise survival for contaminating organisms.

c. Organism survival in the radiation environment adjacent to the target.

d. Probability of encountering/landing on the target, including spacecraft reliability.

e. Probability of surviving landing/impact on the target.

f. Mechanisms and timescales of transport to the subsurface.

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

Note: Parameters for demonstrating contamination avoidance are to be based on current scientific consensus and may require workshops or other non-advocate review panels as per NPR 8715.24.

APPENDIX D. PRE-LAUNCH PP REPORT—EXPECTED ELEMENTS

D.1 Purpose

D.1.1 Provides verification that planetary protection requirements have been met and that the mission has an acceptable risk posture to continue to satisfy planetary protection requirements. This document should minimally provide resolution of all requirements addressed in the PP implementation plan and any additional requirements that have resulted thereafter.

D.1.2 The amount and depth of description will vary with the mission categorization level. The format is guidance only—the mission may elect to generate a word based document to take whatever form is appropriate as long as the information is covered.

D.2 Planetary Protection Pre-Launch Report Outline

D.2.1 Compliance of PP Requirements (e.g., bioburden, inadvertent impact, organic inventory, landing site selection rationale, etc.)

D.2.2 Deviations from the PP Implementation Plan

D.2.3 Summary and resolution of quality reports (anomaly reporting) that impacted PP implementation

D.2.4 Supporting analysis that demonstrates compliance of PP requirements

D.2.5 Considerations that may impact planetary protection compliance

D.2.6 Reviews and Additional Deliverables

Note: Alternatively, prelaunch reports may be organized based on topical requirements such as bioburden and inadvertent impact.

D.3 Planetary Protection Pre-Launch Report Approaches

D.3.1 For Category II, III, & IV missions, updates to the mission design and navigation plan in relation to demonstrating avoidance of contamination of the launched elements leaving Earth's orbit and spacecraft, as applicable.

D.3.2 For Category III & IV missions, status of spacecraft and launch vehicle cleanliness, including reports of current bioburden and implementation of the margin management approach, detailing any remaining work.

D.3.3 Other problem and anomaly events that could impact spacecraft biological, molecular, and particulate cleanliness:

D.3.3.1 Example events include, but are not limited to:

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D.3.3.1.1 Foreign object debris (FOD), including operational debris, environmental debris, and macro-organisms (e.g., birds, reptiles, bats, rodents, insects, spiders, and mites).

D.3.3.1.2 Inadvertent contact of the spacecraft with a surface (e.g., tooling, essential and non-essential personnel, GSE).

D.3.3.1.3 Breach of facility cleanliness control (e.g., medical emergency, equipment failure).

D.3.3.2 Quality reports (anomaly reporting)

D.3.3.2.1 For quality reports, the following are typically included as part of the documentation:

- a. A detailed description of the occurrence
- b. Any impacted hardware and assembly, test, transport, launch environments, or operation impacts. Associated cleanroom particulate and biological verification data with the event (i.e., before, during and after)
- c. Mitigation and resolution pathways
- d. Current state of the system
- e. Any outstanding corrective action and planning items

APPENDIX E. POST-LAUNCH PP REPORT—EXPECTED ELEMENTS

E.1 Purpose

Provides compliance with overall PP requirements as a summary document based on the "Pre-Launch PP Report" but updated to include the effects of launch, early postlaunch events, and any additional requirements that have resulted thereafter.

E.2 Planetary Protection Post-Launch Report Outline

E.2.1 Post-launch events

E.2.2 Launch and Post-Launch summary (e.g. trajectory actual vs. planned, updates to previous documentation)

E.2.3 Compliance of outstanding PP requirements (e.g., final bioburden accounting, inadvertent impact, organic inventory, etc.)

E.2.4 Deviations from the PP Pre-launch Report

E.2.5 Summary and resolution of quality reports (anomaly reporting) that impacted PP implementation

E.2.6 Additional supporting analysis since Pre-launch Report to demonstrate compliance of PP requirements (e.g., launch recontamination analysis, delivery of final bioburden management and accounting data, Planetary Protection Equipment List [PPEL])

Note: The PPEL is a well-known NASA example of acceptable bioburden management accounting when using culture-based spore assay for the bioburden control approach. The PPEL is typically derived from the project's master equipment list.

E.2.7 Reviews and additional deliverables

Note: Alternatively, post-launch reports may be organized based on topical requirements such as bioburden and inadvertent impact.

**APPENDIX F. PP EXTENDED MISSION REPORT—EXPECTED
ELEMENTS**

F.1 Purpose

F.1.1 The PP Extended Mission Report details and updates the project’s plans to remain complaint for PP requirements during the extended mission operations.

F.1.2 Extended Mission Reports may introduce new operations or target bodies that would require additional PP requirements and associated analysis to demonstrate compliance.

F.2 Planetary Protection Extended Mission Report Outline

F.2.1 Summary of extended mission activities

F.2.2 Additional PP requirements, implementation and execution

F.2.3 Compliance of outstanding PP requirements (e.g., inadvertent impact, probability of contamination, updated disposal plans, etc.)

F.2.4 Deviations from prior PP reports

F.2.5 Summary and resolution of quality reports (anomaly reporting) that impacted PP implementation

F.2.6 Additional supporting analysis since Post-launch Report to demonstrate compliance of PP requirements

F.2.7 Reviews, updated PP documentation and additional Deliverables

Note: Alternatively, extended mission reports may be organized based on topical requirements such as bioburden and inadvertent impact.

APPENDIX G. END-OF-MISSION PP REPORT—EXPECTED ELEMENTS

G.1 Purpose

At the formally declared "end-of-mission," PP Category II-V missions submit an End-of-Mission Report which documents the compliance of any open planetary protection requirements and reports the final disposition of all launched hardware (e.g., launch vehicle and mission associated).

G.2 End-of-Mission PP Report Outline

G.2.1 Compliance of Outstanding PP Requirements (e.g., final hardware disposition or impact location)

Note: For Category V (r) missions this includes the details of transferring the samples to an appropriate containment facility.

G.2.2 Deviations from prior PP reports

G.2.3 Summary and resolution of quality reports (anomaly reporting) that impacted PP implementation since prior PP report.

G.2.4 Additional supporting analysis since Post-launch or Extended Mission Report to demonstrate compliance of PP requirements.

APPENDIX H. BIOBURDEN MANAGEMENT AND ACCOUNTING

H.1 Overview

H.1.1 For PP Category III and IV missions, when applicable, a bioburden management and accounting process is necessary for tracking and reporting bioburden. A list of all collected data associated with spacecraft components is updated throughout the project life cycle. The following parameters are used to support the bioburden management and accounting process:

- H.1.1.1 Hardware item and description.
- H.1.1.2 Accountable surface area.
- H.1.1.3 Mated and/or internal surface area.
- H.1.1.4 Non-metallic / encapsulated volumes.
- H.1.1.5 Bioburden allocation value.
- H.1.1.6 Bioburden allocation estimation source (e.g., manufacturing provenance, engineering judgement, etc.).
- H.1.1.7 Bioburden margin.
- H.1.1.8 Hardware microbial reduction applied, if applicable.
- H.1.1.9 Final bioburden value for specific component.