Planetary protection protocols for space exploration: *a practical introduction*

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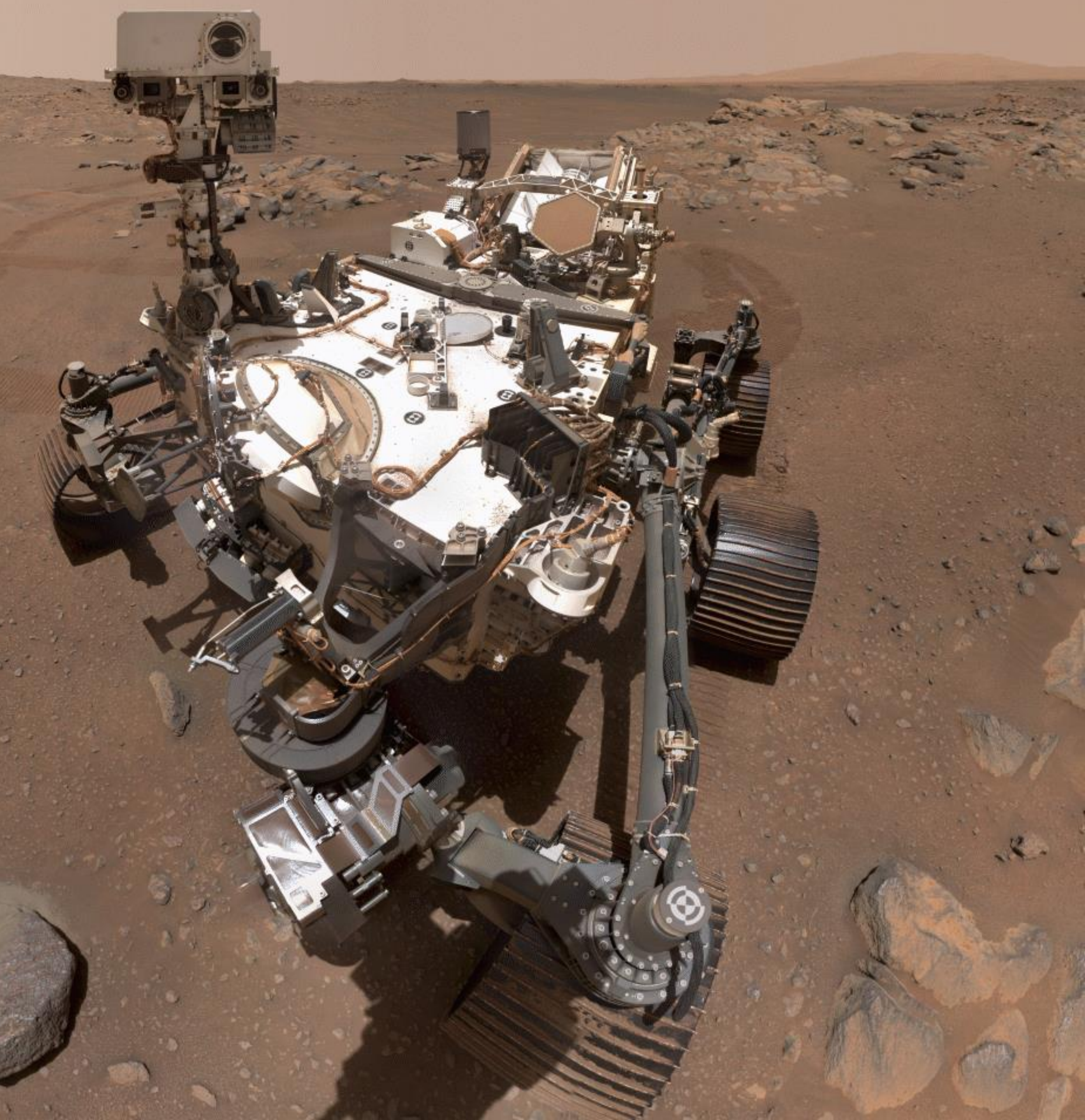
Centro de Astrobiología (CAB), INTA-CSIC, Spain

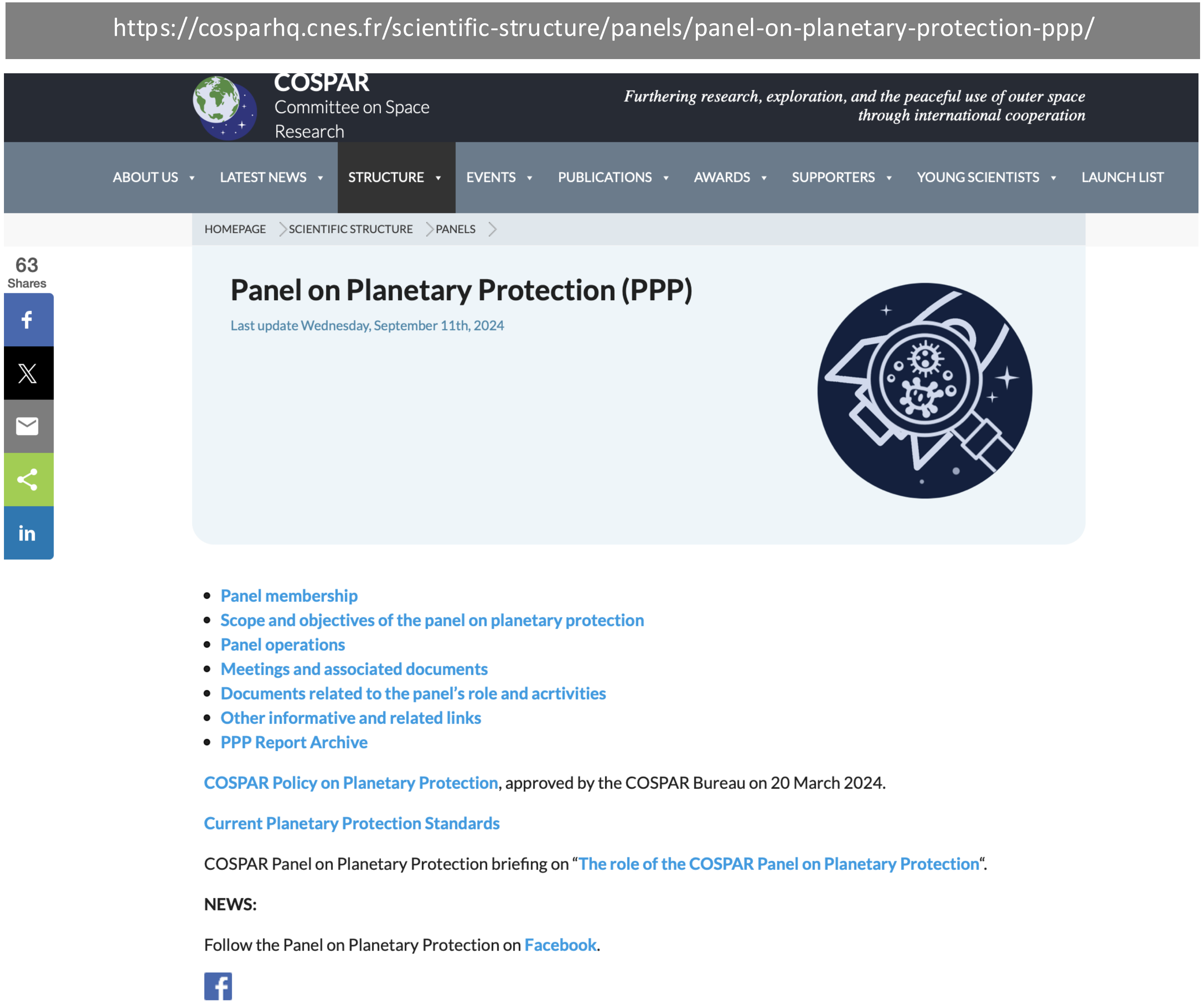
Planetary protection is crucial in space exploration, safeguarding both the integrity of extraterrestrial life investigations and Earth's biosphere from potentially hazardous extraterrestrial material. Current protocols emphasize bioburden reduction and contamination control, requiring rigorous sterilization and detection in cleanroom.
This talk will review these established standards, pinpointing gaps and exploring how advanced molecular techniques, such as metagenomics, can enhance planetary protection standards. Through examples from recent space missions, we will discuss practical applications and assess the potential of new biological tools in this field.



COSPAR

(Committee on Space Research)

Planetary Protection Policy (PPP): is an international voluntary and non-legally binding standard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Furthering research, exploration, and the peaceful use of outer space through international cooperation

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Panel on Planetary Protection (PPP)

Last update Wednesday, September 11th, 2024

- Panel membership
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- Panel operations
- Meetings and associated documents
- Documents related to the panel's role and acrtivities
- Other informative and related links
- PPP Report Archive

COSPAR Policy on Planetary Protection, approved by the COSPAR Bureau on 20 March 2024.

Current Planetary Protection Standards

COSPAR Panel on Planetary Protection briefing on "The role of the COSPAR Panel on Planetary Protection".

NEWS:

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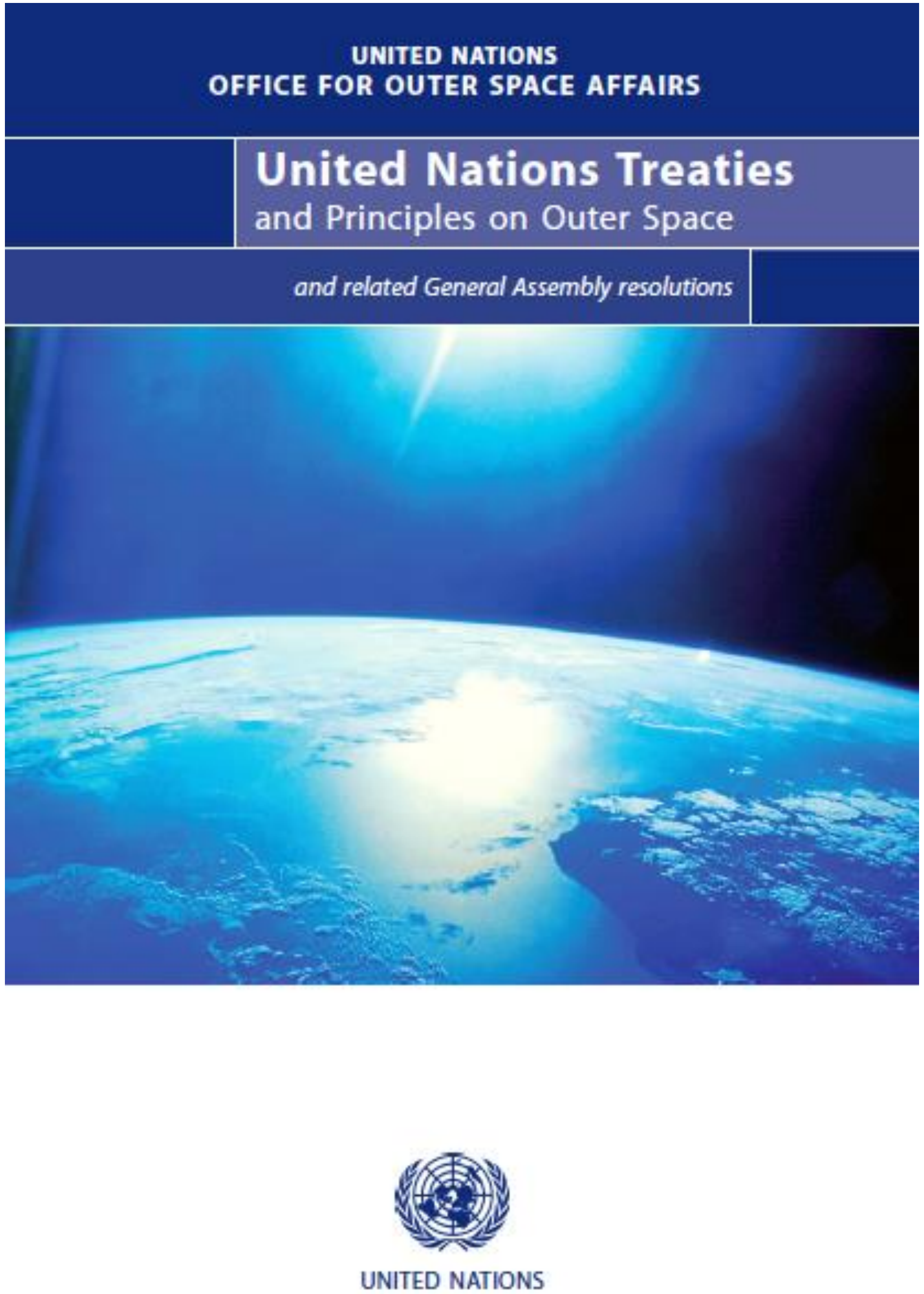


The treaties

Article IX

In the exploration and use of outer space, including the Moon and other celestial bodies, States Parties to the Treaty shall be guided by the principle of cooperation and mutual assistance and shall conduct all their activities in outer space, including the Moon and other celestial bodies, with due regard to the corresponding interests of all other States Parties to the Treaty. States Parties to the Treaty shall pursue studies of outer space, including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose. If a State Party to the Treaty has reason to believe that an activity or experiment planned by it or its nationals in outer space, including the Moon and other celestial bodies, would cause potentially harmful interference with activities of other States Parties in the peaceful exploration and use of outer space, including the Moon and other celestial bodies, it shall undertake appropriate international consultations before proceeding with any such activity or experiment. A State Party to the Treaty which has reason to believe that an activity or experiment planned by another State Party in outer space, including the Moon and other celestial bodies, would cause potentially harmful interference with activities in the peaceful exploration and use of outer space, including the Moon and other celestial bodies, may request consultation concerning the activity or experiment.

Entered into force, Oct. 10th 1967; ~100 signatories

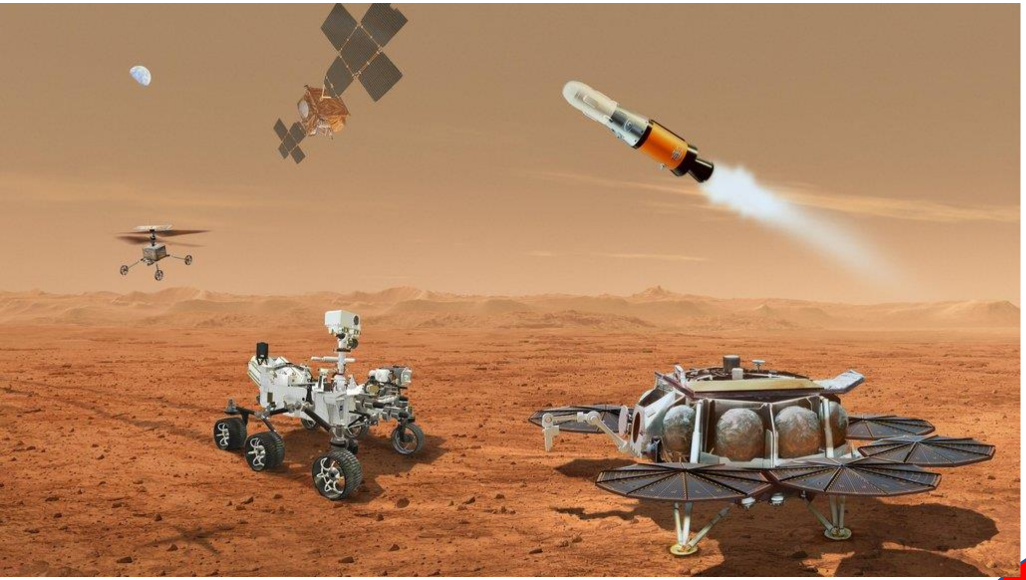
COSPAR Planetary Protection Policy

- The conduct of scientific investigations of possible **extraterrestrial life forms, precursors, and remnants** must no be jeopardized.

- The **Earth must be protected** from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from an interplanetary mission.

- Therefore, for certain space mission/target planet combinations, **controls on contamination** shall be imposed in accordance with issues implementing this policy.

So.. .what is planetary protection? Is the policy and the technical implementations to prevent forward and backward contamination



__Planetary Protection__

Avoiding cross-contamination of biospheres

COSPAR Planetary Protection

‘**COSPAR Policy on Planetary Protection**’. Space Research Today, vol. 220, July. 2024, pp. 10–36.

Coustenis, Athena, et al. ‘Planetary Protection: Updates and Challenges for a Sustainable Space Exploration’. Acta Astronautica, vol. 210, Sept. 2023, pp. 446–52., https://doi.org/10.1016/j.actaastro.2023.02.035.

(Venus) Zorzano, María Paz, et al. ‘The COSPAR Planetary Protection Requirements for Space Missions to Venus’. *Life Sciences in Space Research*, vol. 37, May 2023, pp. 18–24. https://doi.org/10.1016/j.lssr.2023.02.001.

(Mars) Olsson-Francis, Karen, et al. ‘The COSPAR Planetary Protection Policy for Robotic Missions to Mars: A Review of Current Scientific Knowledge and Future Perspectives’. Life Sciences in Space Research, vol. 36, Feb. 2023, pp. 27–35. https://doi.org/10.1016/j.lssr.2022.12.001.

https://cosparhq.cnes.fr/scientific-structure/panels/panel-on-planetary-protection-ppp/

Table 1. Planetary Protection Categories in relation to target bodies.

Category	Mission Type	Target Body
I	Flyby, Orbiter, Lander	Undifferentiated, metamorphosed asteroids; Io; others to-be-defined (TBD)
II	Flyby, Orbiter, Lander	Venus; Moon (Cat. II, IIa & IIb); Comets; Carbonaceous Chondrite Asteroids; Jupiter; Saturn; Uranus; Neptune; Ganymede*; Callisto; Titan*; Triton*; Pluto/Charon*; Ceres; Kuiper-belt objects > ½ the size of Pluto*; Kuiper-belt objects < ½ the size of Pluto; others TBD
III	Flyby, Orbiters	Mars; Europa; Enceladus; others TBD
IV	Landers	Mars (Cat. IVa, IVb, & IVc); Europa; Enceladus; others TBD
V "Restricted Earth return"	-	Mars; Europa; Enceladus; others TBD
V "Unrestricted Earth return"	-	Venus, Moon; others TBD

(Icy Moons) Doran, P. T., et al. ‘The COSPAR Planetary Protection Policy for Missions to Icy Worlds: A Review of History, Current Scientific Knowledge, and Future Directions’. Life Sciences in Space Research, vol. 41, May 2024, pp. 86–99. https://doi.org/10.1016/j.lssr.2024.02.002.

(Human exploration) Spry, James A., et al. ‘Planetary Protection Knowledge Gap Closure Enabling Crewed Missions to Mars’. *Astrobiology*, vol. 24, no. 3, Mar. 2024, pp. 230–74. https://doi.org/10.1089/ast.2023.0092

NASA Interim Directive

NID 8715.129
NRW 1400.132

Effective Date: July 9, 2020
Expiration Date: July 9, 2023

Subject: Biological Planetary Protection for Human Missions to Mars

Responsible Office: Office of Safety and Mission Assurance

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NASA Procedural Requirements

NPR 8715.24
Effective Date: September 24, 2021
Expiration Date: September 24, 2026

COMPLIANCE IS MANDATORY FOR NASA EMPLOYEES

Planetary Protection Provisions for Robotic Extraterrestrial Missions

Responsible Office: Office of Safety and Mission Assurance

NID 8715.129 Biological Planetary Protection for Human Missions to Mars.

Measurement System Identification:	
NASA TECHNICAL STANDARD **National Aeronautics and Space Administration**	**NASA-STD-8719.27**
	Approved: 2022-08-30 **Baseline**

IMPLEMENTING PLANETARY PROTECTION REQUIREMENTS FOR SPACE FLIGHT

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ECSS | European Cooperation for Space Standardization

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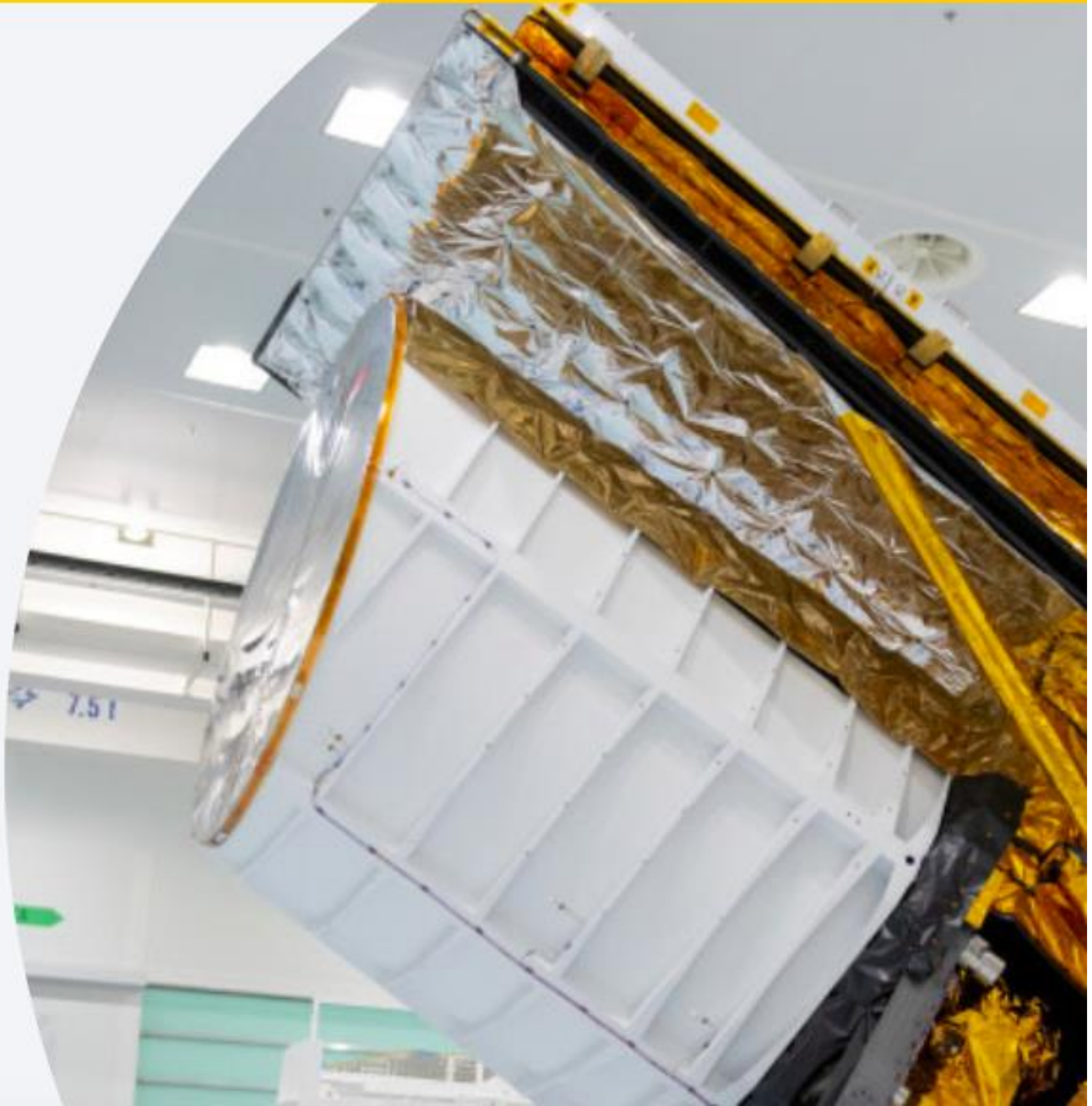
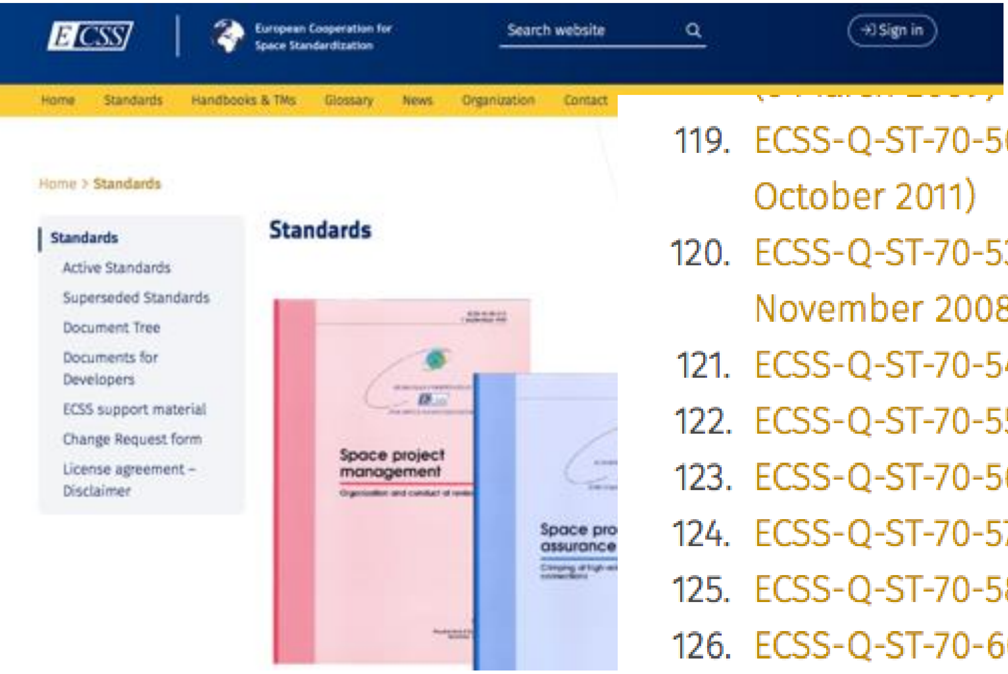


https://ecss.nl



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https://ecss.nl

119. ECSS-Q-ST-70-50C – Particles contamination monitoring for spacecraft systems and cleanrooms (4 October 2011)
120. ECSS-Q-ST-70-53C – Materials and hardware compatibility tests for sterilization processes (15 November 2008)
121. ECSS-Q-ST-70-54C – Ultracleaning of flight hardware (15 February 2017)
122. ECSS-Q-ST-70-55C – Microbial examination of flight hardware and cleanrooms
123. ECSS-Q-ST-70-56C – Vapour phase bioburden reduction for flight hardware (30 August 2013)
124. ECSS-Q-ST-70-57C: Dry heat bioburden reduction for flight hardware (30 August 2013)
125. ECSS-Q-ST-70-58C – Bioburden control of cleanrooms (15 November 2008)
126. ECSS-Q-ST-70-60C Corrigendum 1 – Qualification and procurement of printed circuit boards (1 March 2019)
127. ECSS-Q-ST-70-71C Rev.1 – Materials, processes and their data selection (15 October 2019)
128. ECSS-Q-ST-70C Rev.2 – Materials, mechanical parts and processes (15 October 2019)
129. ECSS-Q-ST-80C Rev.1 – Software product assurance (15 February 2017)
130. ECSS-S-ST-00-01C – Glossary of terms (1 October 2012)
131. ECSS-S-ST-00C – Description, implementation and general requirements (31 July 2008)
132. ECSS-U-AS-10C Rev.1 – Adoption Notice of ISO 24113: Space systems – Space debris mitigation requirements (3 December 2019)
133. ECSS-U-ST-20C – Space sustainability – Planetary protection (1 August 2019)

ECSS-U-ST-20C
1 August 2019

EUROPEAN COOPERATION
ECSS
FOR SPACE STANDARDIZATION

Space sustainability

Planetary protection

ECSS Secretariat
ESA-ESTEC
Requirements & Standards Division
Noordwijk, The Netherlands

- Planetary Protection
- STANDARD

Foreword

This Standard is one of the series of ECSS Standards intended to be applied together for the management, engineering, product assurance and sustainability in space projects and applications. ECSS is a cooperative effort of the European Space Agency, national space agencies and European industry associations for the purpose of developing and maintaining common standards. Requirements in this Standard are defined in terms of what shall be accomplished, rather than in terms of how to organize and perform the necessary work. This allows existing organizational structures and methods to be applied where they are effective, and for the structures and methods to evolve as necessary without rewriting the standards.

This Standard has been prepared by the ECSS-U-ST-20C Working Group, reviewed by the ECSS Executive Secretariat and approved by the ECSS Technical Authority.

This standard describes the planetary protection requirements for spaceflight missions based on the COSPAR planetary protection policy and requirements. The content of this document has been coordinated with the already existing ESA and NASA standards to ensure that requirements, documentation and reviews cover the needs and obligations of international partners for joint missions or contributions to a third party mission.

ECSS-S-ST-00-01: ECSS System

Glossary of terms

ECSS-Q-ST-10-09: Space product assurance

Non-conformance control system

ECSS-Q-ST-40: Space product assurance

Safety

ECSS-Q-ST-70-01: Space product assurance

Cleanliness and contamination control

ECSS-Q-ST-70-53: Space product assurance –

Materials and hardware compatibility tests for sterilization processes

ECSS-Q-ST-70-55: Space product assurance –

Microbial examination of flight hardware and cleanrooms

ECSS-Q-ST-70-56: Space product assurance –

Vapour phase bioburden reduction of flight hardware

ECSS-Q-ST-70-57:Space product assurance –

Dry heat bioburden reduction of flight hardware

ECSS-Q-ST-70-58: Space product assurance –

Bioburden control of cleanrooms

Credit: ExoMars rover ESA.
Airbus Space, Stevenage (UK), Airbus, Toulouse (France) and Thales Alenia Space, Cannes (France)

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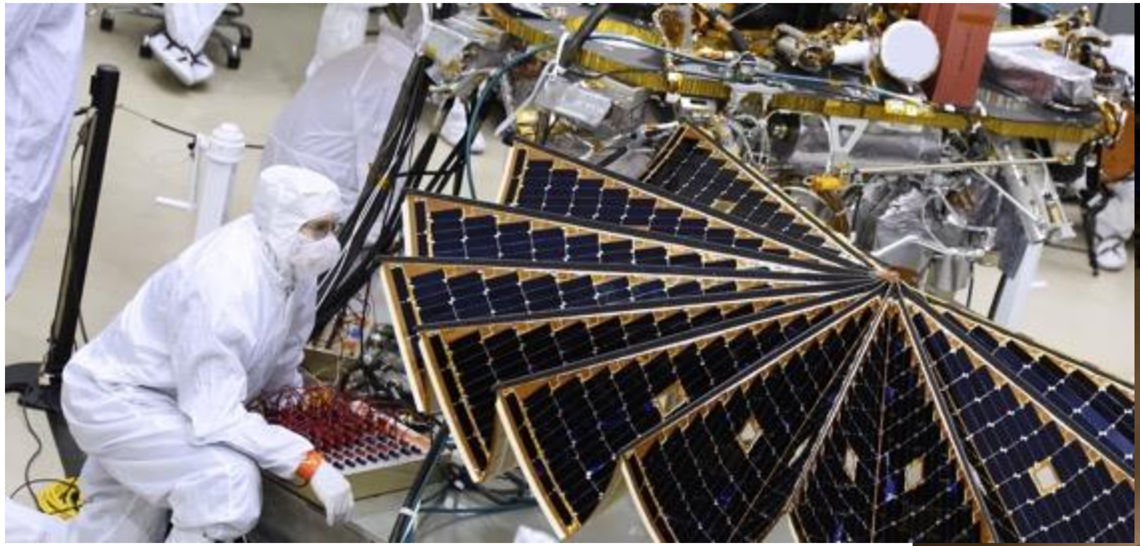
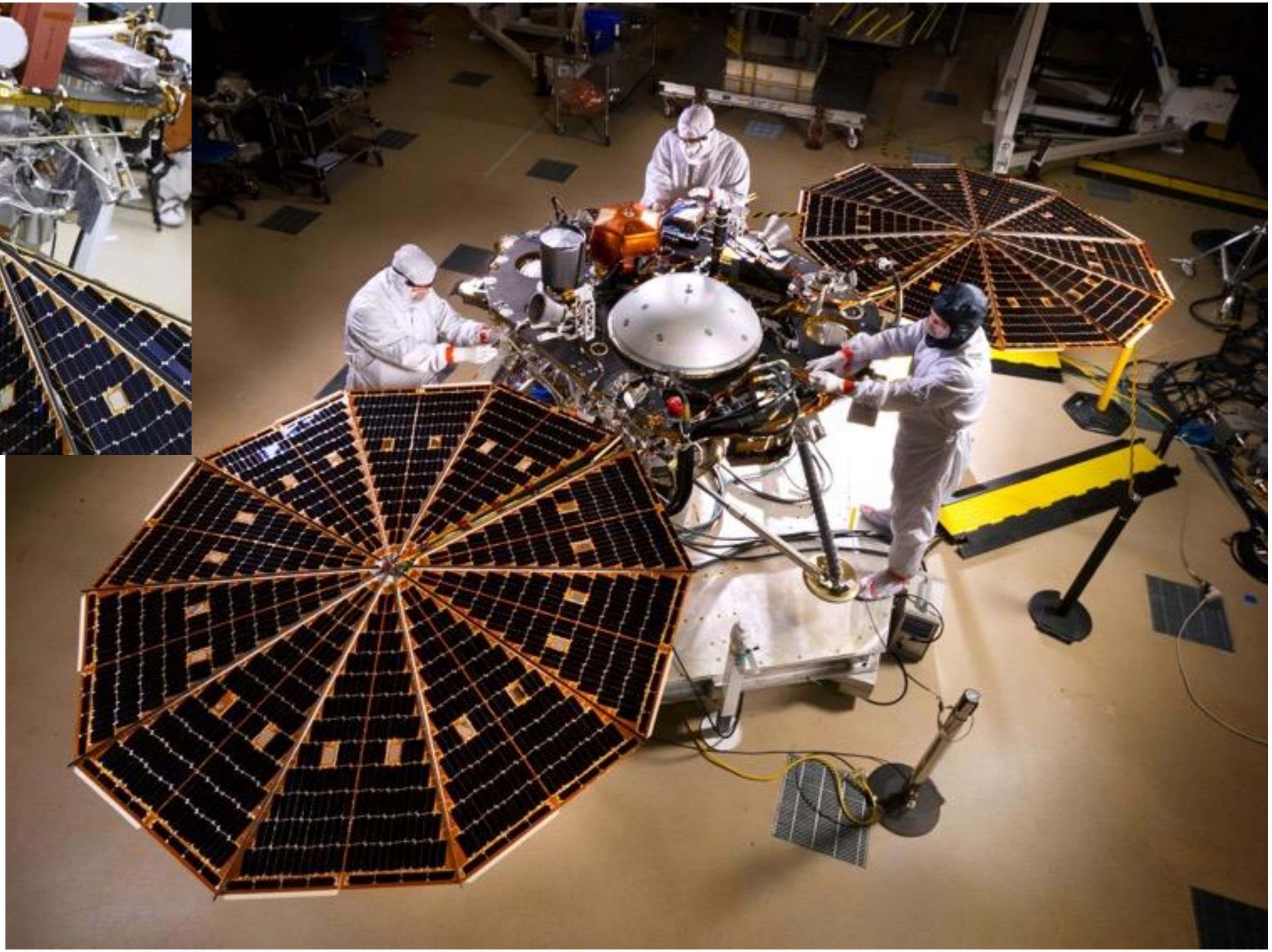
Credit: NASA, Mars Exploration Rovers

- Requirements to carry a total of no more than 300,000 bacterial spores on any surface from which the spores could get into the martian environment.

Credit NASA, JPL-Caltech. InSight Lander. Solar Panels and Planetary Protection





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Credit NASA, Sample Caching System (carousel) in the Mars 2020 rover

The bit carousel, which lies at the heart of Sample Caching System of NASA's Mars 2020 mission, is attached to the front end of the rover in the Spacecraft Assembly Facility's High Bay 1 at the Jet Propulsion Laboratory in Pasadena, California. The carousel contains all of the tools the coring drill uses to sample the Martian surface and is the gateway for the samples to move into the rover for assessment and processing.

Clean Room and HEPA filters

HEPA filters, as defined by the United States Department of Energy (DOE) standard adopted by most American industries, **remove at least 99.97% of airborne particles 0.3 micrometers (µm) in diameter**. The filter's minimal resistance to airflow, or pressure drop, is usually specified around 300 pascals (0.044 psi) at its nominal flow rate. The specification usually used in the European Union is the European Norm EN 1822:2009.

Clean rooms usually have a small over pressure.
Particle Counters are used to certify the ISO of a clean room.

Clean rooms require the use of special protective garment.

Clean rooms are used for assembly of electronic/optic components, pharmaceutical and food industry, microbiology environments, and space technology assembly.

Credit: https://www.cleanroom-industries.com/index.php/resources/cleanroom-hepa-filters-specifications/298

ECSS-U-ST-20C
1 August 2019

EUROPEAN COOPERATION FOR SPACE STANDARDIZATION

ECSS

Space sustainability

Planetary protection

Credit: AI generated. TheDigitalArtist

Table 5-1: Bioburden estimation

Bioburden type	Specific environment	Bioburden value
Average encapsulated spores density (i.e. if no differentiation between electronic and non-electronic piece parts is made)	Non-metallic parts of the spacecraft:	130 spores/cm³
Source specific encapsulated spore density	Electronic piece parts:	3-150 spores/cm³
	Other non-metallic materials:	1-30 spores/cm³
Source specific enclosed surface spore density, e.g. a box closed in the specific environment	ISO class 8 cleanroom, highly controlled:	500-5000 spores/m²
	ISO class 8 cleanroom, normally controlled:	5000-10^5 spores/m²
	Uncontrolled environment:	10^5-10^6 spores/m²
Average surface spore density for cleanroom classes "in operation" (exposed and mated but non-encapsulated)	ISO class 7 cleanroom or better, highly controlled:	50 spores/m²
	ISO class 7 cleanroom or better, normally controlled:	500 spores/m²
	ISO class 8 cleanroom, highly controlled:	1 000 spores/m²
	ISO class 8 cleanroom, normally controlled:	10 000 spores/m²
	Uncontrolled environment:	10^5 spores/m²

NOTE 1: Manufacturing processes can potentially be used to claim a lower encapsulated bioburden either through high temperatures (see ECSS-Q-ST-70-57 for relevant specifications) or control of manufacturing environment.

NOTE 2: Normally controlled: use of gowning equivalent to the specific cleanroom class.

NOTE 3: Highly controlled: bioburden control of cleanroom by use of full body coverall, hood, face mask, gloves and boots, restricted access and dedicated cleaning and microbial sampling.

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ECSS-Q-ST-70-58C Bioburden control of **cleanrooms**

- The objective of this Standard is to ensure that the proper procedures to control the microbiological contamination in controlled environments are in place to meet the planetary protection constraints.
- **aseptic** state of being free from all living microorganisms (i.e. free of bioburden) NOTE In practice, it is usually described as a probability.
- **biobarrier(s)** barrier surrounding an item which prevents biological recontamination subsequent to microbial reduction procedures
- **bioburden** quantity of viable microorganisms measured with a specified assay
- **bioburden reduction** process or processes used to reduce the viable microbial population on an item to an acceptable limit
- **controlled environment** defined zone in which contamination is controlled by specified means.
- **sporicide** substance capable of destroying bacterial spores
- **sterilization** validated process used to render product free from viable micro-organisms (ISO 11139)

- **The Planetary Protection of Outer Solar System (PPOSS) Project**
- *The PPOSS project Coordination and Support Action has received funding from the European Commission's H2020 Program (2016 -2018) under grant agreement 687373. -- pdf in EU portal*
- http://pposs.org

STERILIZATION STANDARDS

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

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

BIOBURDEN ON SPACE CRAFT

Type	Methods	Sterilization type		Heritage	
		Surface	Bulk	Studied	Studied and used
CHEMICAL	Formaldehyde gas	X	--	Space components (US 1968)	--
	Ethylen oxide (EtO)	X	--	--	Ranger 1961/62
	Sporicidal solution (TBD)	X	--	Mars 96	Mariner Mars 1971
	Hydrogen peroxide	X	--	--	Mars96, Beagle2, DS2
THERMAL	Dry Heat	X	X	--	Viking, Mars96, Pathfinder, Beagle2, MER, Phoenix, MSL
STEAM	Steam (space hardware excluded)	X	--	--	Excluded on space h/w, only GSE, garments
RADIATIVE	Gamma / Beta radiations	X	X	--	Mars96, Beagle2

Table 6 – Bioburden reduction type and mission examples

ECSS-Q-ST-70-57C **Dry heat** bioburden reduction for flight hardware

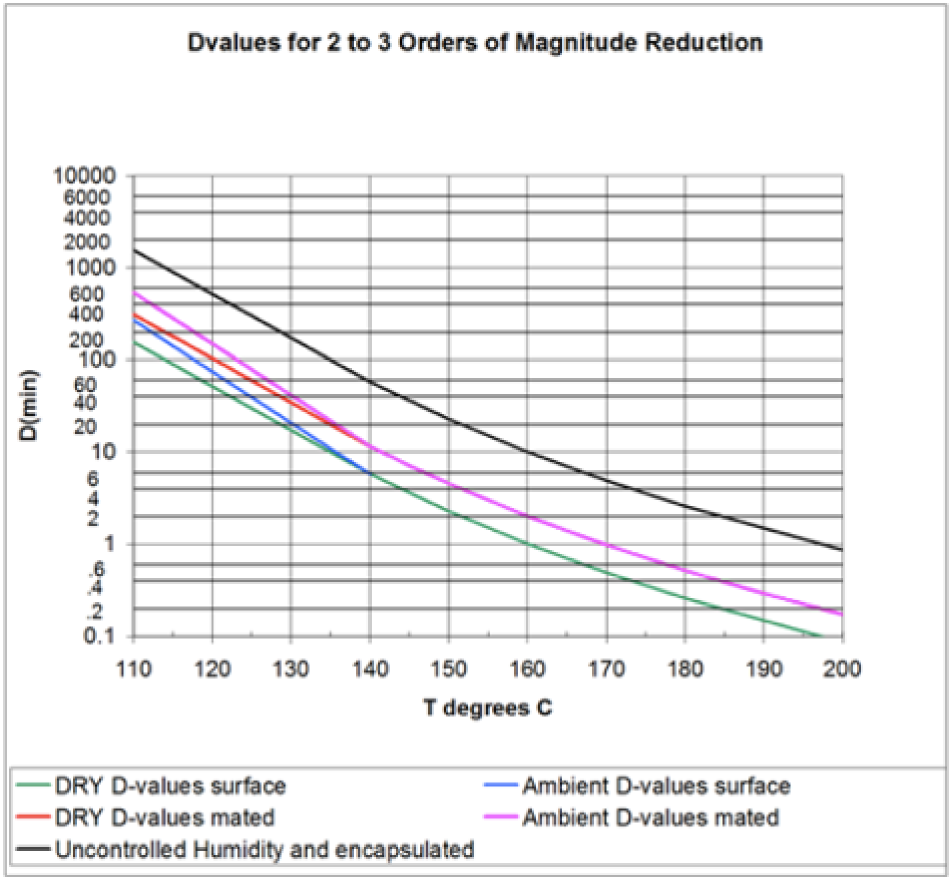

Figure D-1: D-values for 2 to 3 orders of magnitude reduction

Several days at 125°C. ExoMars Parachute
Credit ESA. ExoMars 2022 and Planetary Protection.
It is at least 10 000 times cleaner than your smartphone (4-log reduction)

Credit ESA/ Parachute. ExoMars 2022 and Planetary Protection

The parachute was heated in an oven at 125°C for several days to kill any microbes. The oven is part of ESA's Life, Physical Sciences and Life Support Laboratory, a state-of-the-art facility in the Netherlands. The Laboratory has also cleaned ExoMars instruments and subsystems, but this second stage parachute is the largest item to be sterilised.

The dry heat steriliser is in the **'ISO Class 1'** cleanroom, one of the cleanest places in Europe. All air passes through a two-stage filter ensuring less than 10 dust particles no larger than 10 millionth of a metre, or less than the size of the **coronavirus.**

ECSS-Q-ST-70-55C **Microbial examination** of flight hardware and cleanrooms

- The following test methods are described:
- Surface and air sampling and detection of biological contaminants with swabs, wipes, contact plates and air samplers, followed by **cultivation** for bioburden determination
- Sampling of biological contaminants by DNA analysis from swabs and wipes. The test methods described in this standard apply to controlling the microbiological contamination on all manned and unmanned spacecraft, launchers, payloads, experiments, ground support equipment, and cleanrooms with planetary protection constraints

Notes

- Surfaces with an area of maximal 25 cm^2 shall be sampled with **swabs** and surfaces with an area of maximal 1 m^2 shall be sampled with **wipes** *NOTE: Multiple swabs and wipes can be used to sample a larger surface area*
- Bioburden shall be determined with assays for quantification of aerobic mesophilic bacteria *NOTE: This sampling is only appropriate for materials and material combinations (e.g. electrochemical compatibility, see ECSS-Q-ST-70-71) that can tolerate sample collection using damp materials*

With this assay mesophilic aerobic spores and bacteria that are able to survive a heat treatment for 15 min at 80 °C are determined. The flow-chart for the swab assay 1 is schematically shown in Figure D-1.

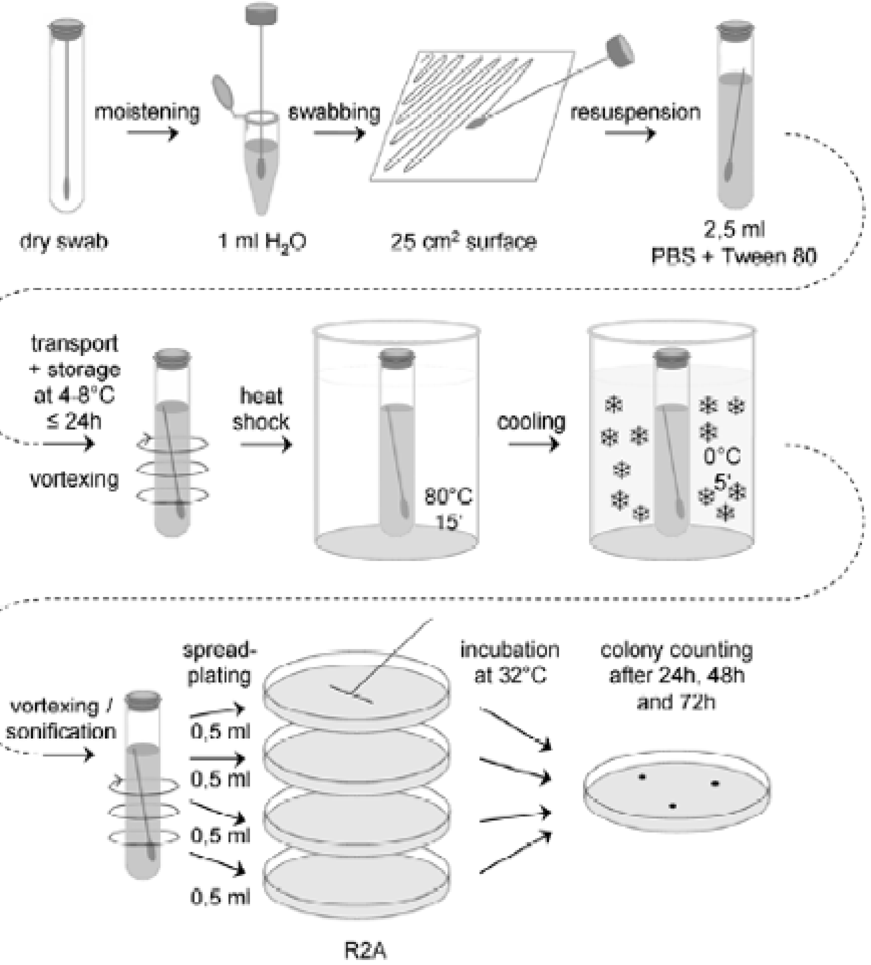

Figure D-1: Flow chart for the standard swab assay (swab assay 1)

With this assay aerobic mesophiles are determined. The flow-chart for the swab assay 2 is schematically shown in Figure D-2.

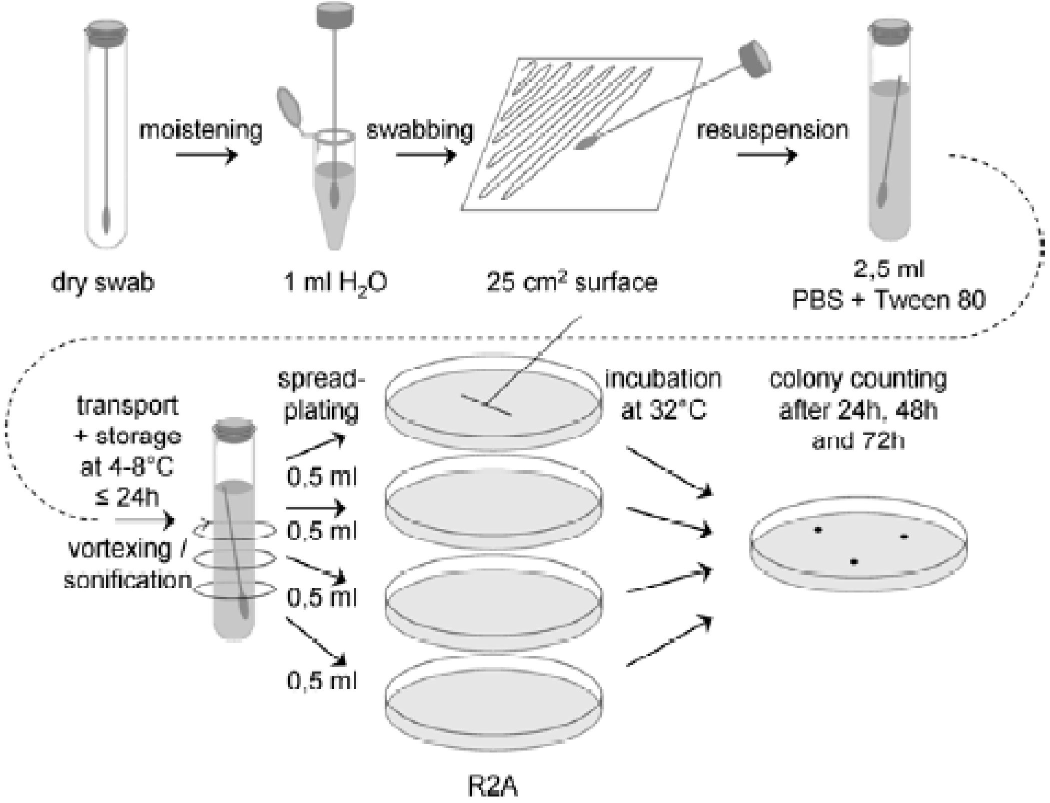

Figure D-2: Flow chart for swab assay 2

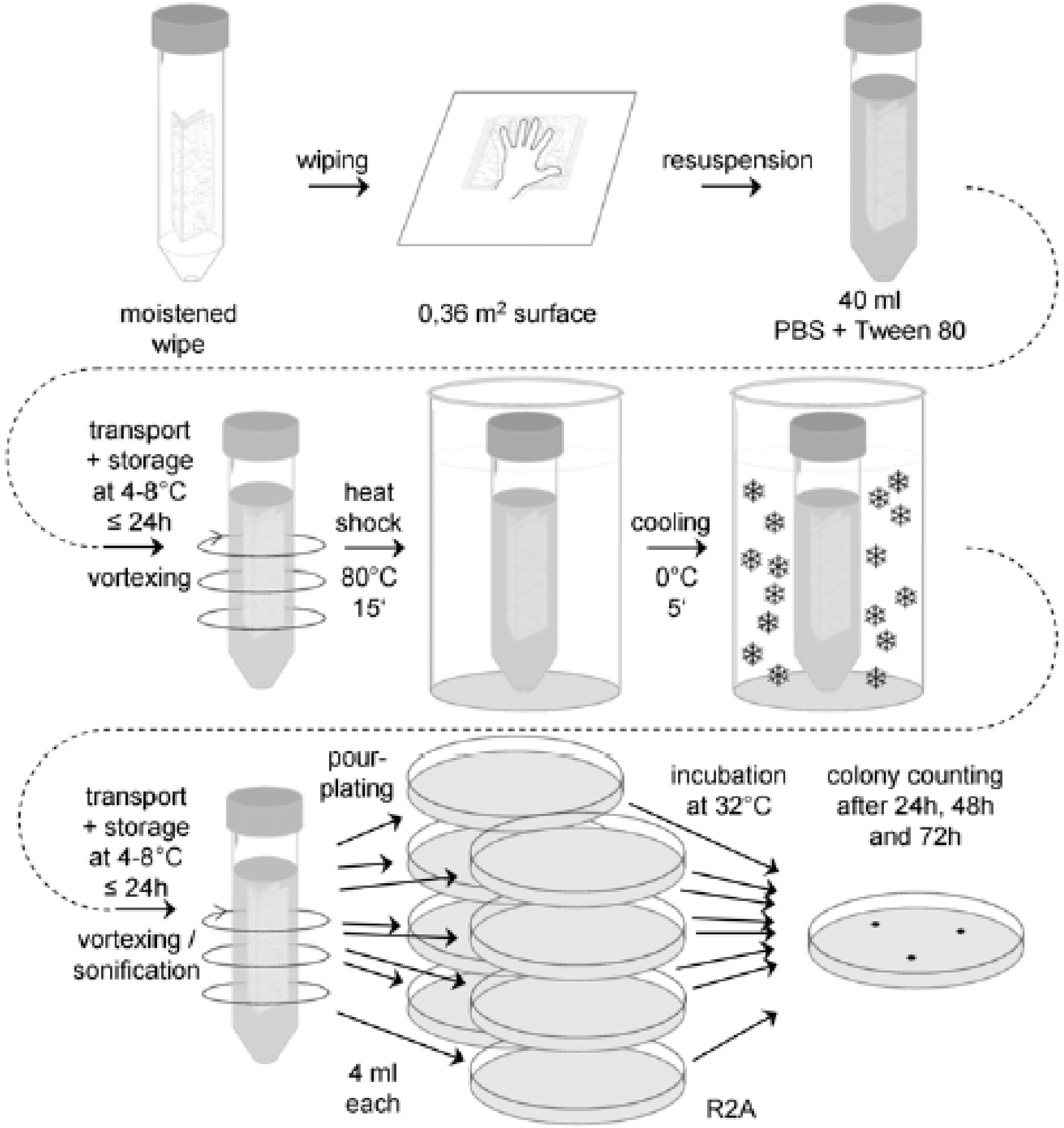

Figure E-1: Flow chart for the standard wipe assay (wipe assay 1)

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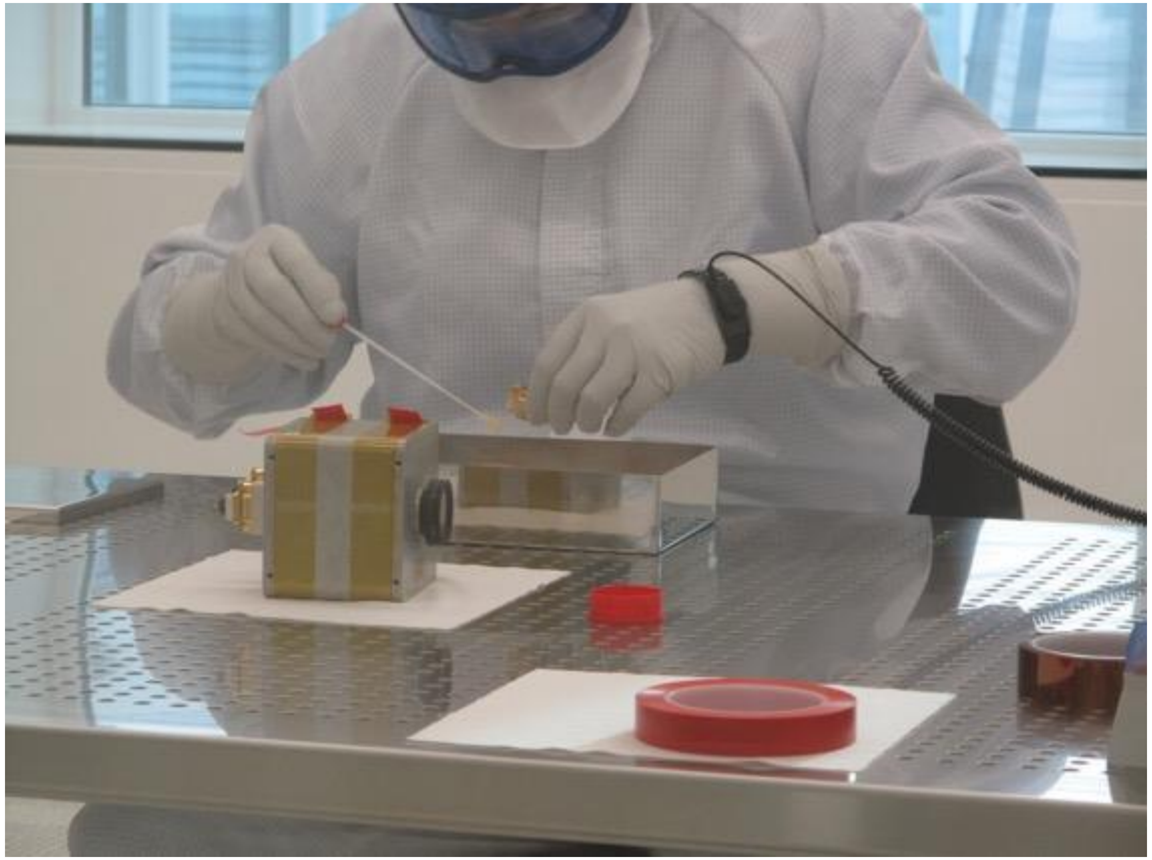
Credit ESA/ Baikonur. Schiaparelli and Planetary Protection

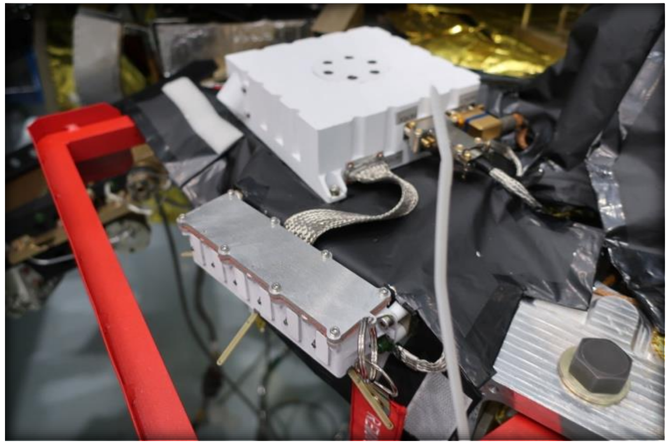
Taking microbiological samples from Schiaparelli, inside the portable cleanroom tent, at Baikonur.*Credit: ESA*

Credit ESA/ Noordwijk. Schiaparelli.

Final assays of ExoMars 2016 Descent Camera flight unit, provided by the ESA Scientific Support Office, in ESA's bioburden-controlled Life and Physical Sciences, Exploration and Life Support Laboratory.

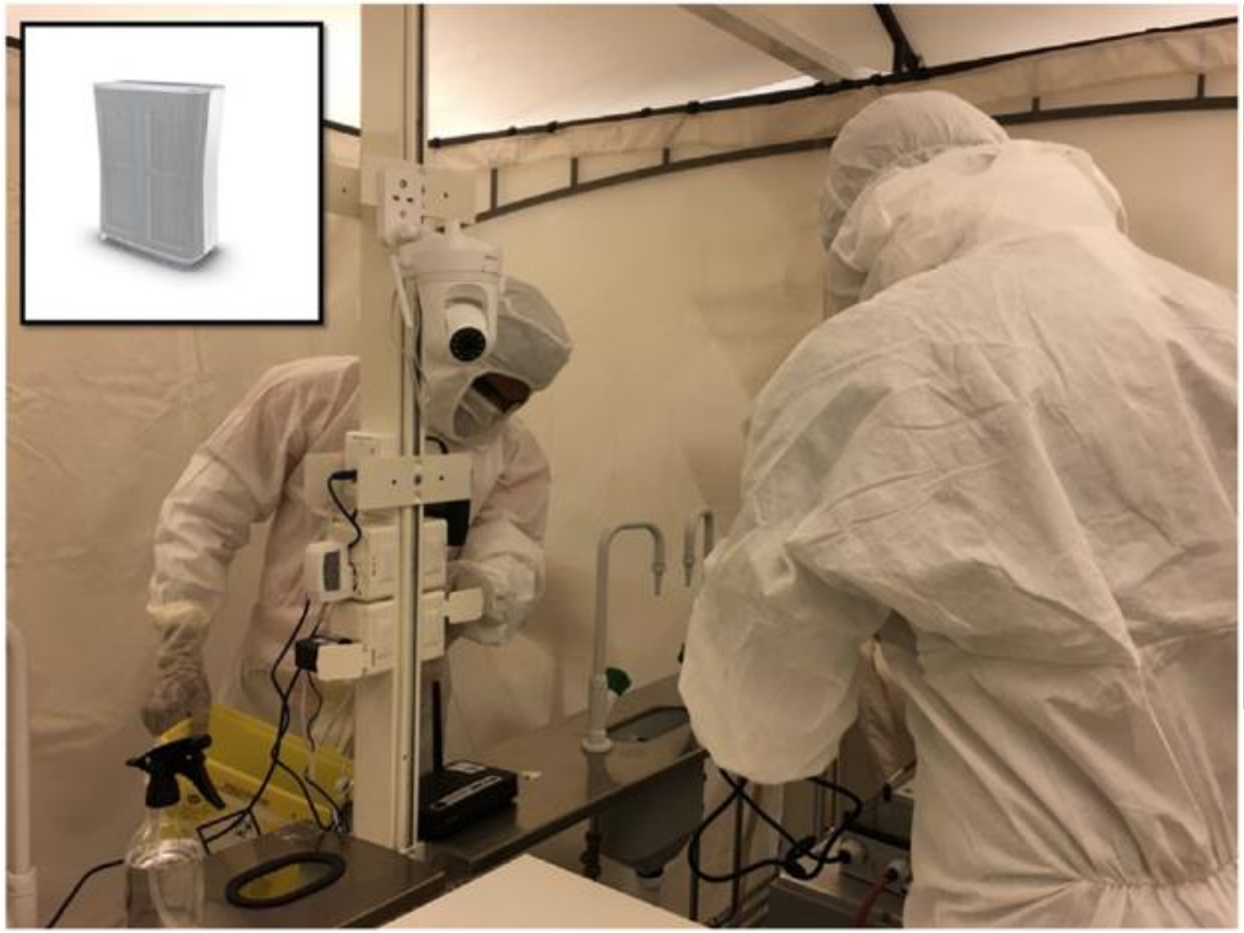


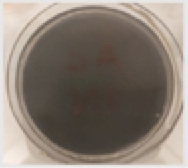
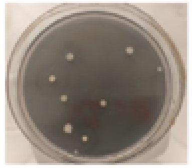
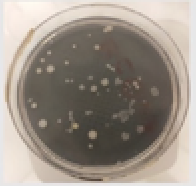
Credits: Thasshwin Mathanlal, Miracle Israel Nazarious, Abhilash Vakkada Ramachandran, Maria-Paz Zorzano, Javier Martin-Torres, Petra Rettberg, 2020, ***Implementing bioburden reduction and control on the deliquescent hydrogel of the HABIT/ExoMars 2020 instrument,*** Acta Astronautica. DOI: 10.1016/j.actaastro.2020.04.030



HABIT, ExoMars Surface Platform (ESA/IKI)

Credits: Thasshwin Mathanlal, Miracle Israel Nazarious, Abhilash Vakkada Ramachandran, Maria-Paz Zorzano, Javier Martin-Torres, Petra Rettberg, 2020, ***Implementing bioburden reduction and control on the deliquescent hydrogel of the HABIT/ExoMars 2020 instrument,*** Acta Astronautica. [DOI: 10.1016/j.actaastro.2020.04.030](https://doi.org/10.1016/j.actaastro.2020.04.030)



Sample	24hr count	48hr count	72hr count	Picture after 72hrs
Inside Tent – Everyday Cleaning	0	0	0	
Inside Tent- Alternate day Cleaning	3	5	8	
Outside Tent	9	16	32	

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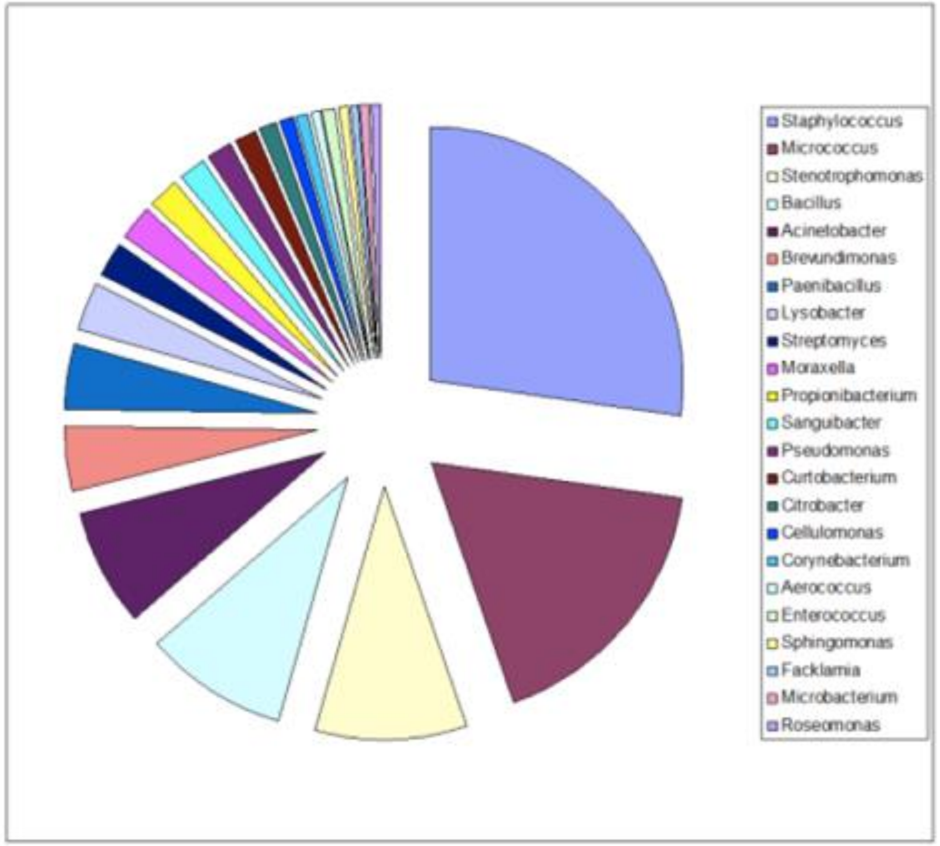
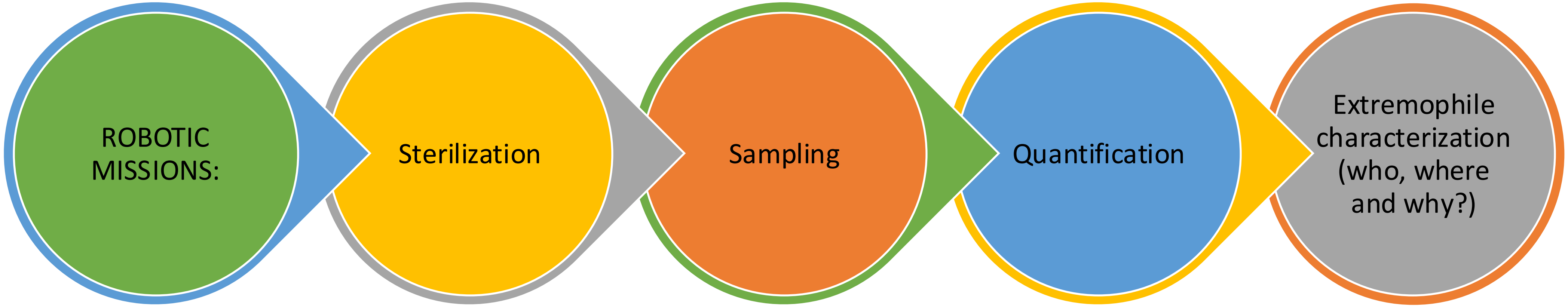


Figure 20 – Example for cultivable diversity at genera level in cleanrooms (Courtesy C. Moissl).

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

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

Planetary protection metagenomics research



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- Microorganisms are everywhere and are smaller than 1 μm.
- They can survive extremely harsh conditions and some of them can form **spores** which are resistant to many physical and chemical stressors.
- Most microbial contaminants in spacecraft assembly cleanrooms are human associated.
- **Current culture-based approaches are biased and insufficient for future exploration targets and missions: only about 1% of all microorganisms are cultivable in a laboratory!!** And we use petri dishes with agar to grow colonies and use them as a proxy to monitor the other remaining 99%.
- The procedures for bioburden control, sterilization and requirements for space exploration missions are **defined in standards**. The missions are classified depending on both their targets of exploration and the mission architecture plan, and this provides an **upper bound of allowed spores per surface/volume.**
- **The standards and the requirements are updated over time depending on the state of the art of scientific knowledge and the technologies.**
- The COSPAR PP Panel updates the policy. NASA, ESA and other space agencies, follow similar procedures.

Planetary protection protocols for space exploration: *a practical introduction*

How can metagenomic methods be useful in the lowest possible biomass limit?

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Low Biomass session

Christopher E. Carr	Georgia Institute of Technology	Low Biomass Metagenomics and Implications for Planetary Protection
Sophie A. Simon	University of Duisburg-Essen	Low-biomass Nanopore metagenomics
Scott Tighe	University of Vermont	Requirements for Assessing Ultra-Low Biomass for Genomic Analysis
Noah Fierer	University of Colorado Boulder	Exploring the limits of microbial life in Antarctic soils
Jyothi Basapathi Raghavendra	University of Aberdeen, UK	DNA sequencing at picogram level for extremely low biomass detection