# ExoMars

## Planetary Protection Requirements

EXM-MS-RS-ESA-00005

Issue 3, Rev. 1

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## DOCUMENT CHANGE LOG

<table>
<thead>
<tr>
<th>Issue/Revision</th>
<th>Date</th>
<th>Modified pages</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/0</td>
<td>28/06/07</td>
<td>All</td>
<td>Update post SRR and IRev</td>
</tr>
</tbody>
</table>
Table of Contents

1 DOCUMENT SCOPE .............................................................................................................................................................5

2 INTRODUCTION....................................................................................................................................................................6

3 REFERENCE DOCUMENTS................................................................................................................................................7

3.1 Normative Documents .......................................................................................................................................................7

3.2 Informative Documents......................................................................................................................................................7

4 EXOMARS PLANETARY PROTECTION CATEGORY ...........................................................................................................9

5 PLANETARY PROTECTION MANAGEMENT ..................................................................................................................10

6 PLANETARY PROTECTION REQUIREMENTS ..................................................................................................................12

6.1 General Mission Requirements .......................................................................................................................................12

6.2 Requirements for the Launch Vehicle .............................................................................................................................13

6.3 Requirements for the Carrier Module .............................................................................................................................13

6.4 Requirements for the Landed System .............................................................................................................................14

7 METHODS AND PROCEDURES ......................................................................................................................................16

7.1 Bioburden Level Estimate ................................................................................................................................................16

7.2 Bioburden Assessment Procedures ................................................................................................................................16

7.3 Bioburden Reduction Procedures ................................................................................................................................18

8 PLANETARY PROTECTION DOCUMENTATION AND REVIEWS .................................................................................20

8.1 Planetary Protection Plan ...............................................................................................................................................20

8.2 Planetary Protection Implementation Requirements .......................................................................................................21

8.3 Planetary Protection Implementation Plan .....................................................................................................................21

8.4 Pre-Launch Planetary Protection Report .......................................................................................................................27

8.5 Post-Launch Planetary Protection Report .......................................................................................................................27

8.6 Mission Extension Planetary Protection Report ............................................................................................................28

8.7 End of Mission Planetary Protection Report ..................................................................................................................28

9 NON-CONFORMANCES AND WAIVERS ............................................................................................................................29

10 LIST OF ACRONYMS AND ABBREVIATIONS ....................................................................................................................32
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>TERMINOLOGY</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>SUMMARY OF DOCUMENTATION AND REVIEWS (NORMATIVE)</td>
<td>35</td>
</tr>
<tr>
<td>A2</td>
<td>ASSAY PROCEDURES (NORMATIVE)</td>
<td>36</td>
</tr>
<tr>
<td>A3</td>
<td>DRY HEAT MICROBIAL REDUCTION PROCEDURE (NORMATIVE)</td>
<td>45</td>
</tr>
<tr>
<td>A4</td>
<td>BIOBURDEN CONTROL IN CLEANROOMS</td>
<td>46</td>
</tr>
<tr>
<td>A5</td>
<td>GUIDELINES FOR TRAINING (INFORMATIVE)</td>
<td>58</td>
</tr>
<tr>
<td>A6</td>
<td>GUIDELINES FOR MEDICAL SCREENING (INFORMATIVE)</td>
<td>59</td>
</tr>
</tbody>
</table>
1 DOCUMENT SCOPE

This document defines the Planetary Protection Requirements for the ExoMars mission as defined in [NR 1]. It is applicable to:

- The ExoMars mission;
- All ExoMars spacecraft elements (carrier module, descent module, rover module, payload, separable equipment, etc.);
- The ExoMars launch vehicle(s).
2 INTRODUCTION

The Outer Space Treaty of 1967 sets up the general principles applicable to the exploration and use of outer space. Article IX of the Outer Space Treaty constitutes the primary statement of international law: “States parties 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, when necessary, adopt appropriate measures for this purpose”. Harmful contamination in that sense is defined as biological contamination, including organic-constituents, to protect the environment and to allow future exobiology research. The Committee On Space Research (COSPAR) has established planetary protection guidelines, based on the Outer Space Treaty. These guidelines impose requirements on spaceflight missions according to 5 categories of target body/mission type combinations [IR 1].

The implementation of planetary protection requirements, particularly for ExoMars, comprise restrictions on impact probabilities for hardware not intended to directly contact Mars, and bioburden control for all spacecraft elements.
3 REFERENCE DOCUMENTS

The documents listed here are available on request to the ExoMars Project Office. In case of conflict between Normative Document and this document the contractor shall inform the ExoMars Project Office for resolution.

3.1 Normative Documents

Normative References are directly applicable in their entirety to this document and are listed below as dated or undated references. These normative references may be cited at appropriate places in the text. For dated references, subsequent amendments to or revisions of any of these document apply to this document only when incorporated in it by amendment or revision. For undated references, the latest signed version is applicable and must be incorporated by the contractor in the project baseline.

[NR 3] ISO 14644-1, Cleanrooms and Associated Controlled Environments – part 1: Classification of air cleanliness.

3.2 Informative Documents

Informative References are applicable to this document only when specifically called up in the text with specific indications of the parts of the document that are to be applicable. Otherwise the documents are listed below for information only as an aid for the purpose of understanding. For dated references, subsequent amendments to or revisions of any of these document apply to this statement of work only when incorporated in it by amendment or revision. For undated references, the latest signed version is applicable and must be incorporated by the contractor in the project baseline pending the use of the document as explained above.

[IR 4] NASA NPG 5340.1D NASA Standard procedures for the microbiological examination of space hardware.
[IR 10] ISO 14698-1, Cleanrooms and Associated Controlled Environments – Biocontamination control: General principles and methods.


[IR 12] ISO 14698-3, Cleanrooms and Associated Controlled Environments – Biocontamination control: Measurement of the efficiency of processes of cleaning and/or disinfection of inert surfaces bearing biocontaminated wet soiling or biofilms.

4 EXOMARS PLANETARY PROTECTION CATEGORY

The ExoMars planetary protection classification based on the mission objectives specified in [NR 1] is in agreement with the COSPAR Planetary Protection Policy [IR 1].

The ExoMars mission is classified as Planetary Protection Category IVb.

C: Planetary protection category IVb is for landed systems with life-detection experiments. The ExoMars mission is not intending to access a Mars special region. See also recommendation of the 2nd ESTAG meeting, 25th of April 2007: “On the basis of the results of the MEPAG Special Regions Science Advisory Group analysis available today [IR 3], ESTAG considers that is possible to fulfil the scientific and technological goals of ExoMars with a site selection process which does not include designated Special Regions as candidate landing sites.”
5 PLANETARY PROTECTION MANAGEMENT

PP-01 The industrial prime contractor shall nominate a Prime Planetary Protection Engineer (P-PPE).

PP-02 The P-PPE shall implement the planetary protection program inside the industrial project team, including subcontractors, instrument providers, and launch service provider. This shall include, but is not limited to:

- Planetary protection implementation requirements for all sub-contractors, including instrument providers and launch service provider;
- Monitoring of the implementation of all planetary protection requirements at sub-contractor, instrument provider, and launch service provider level;
- Specifications, requirements, and commissioning for bioburden controlled facilities;
- General facilities and equipment to meet the planetary protection requirements;
- Medical screening of all personnel identified to work in a bioburden controlled environment;
- Training of all personnel identified to work in a bioburden controlled environment;
- Identification of ITAR and IPR issues specifically related to materials and processes for delivered hardware with respect to bioburden and molecular contamination control.

PP-03 The P-PPE shall prepare and provide all relevant and required documents and analysis related to planetary protection.

C: See chapter 8 for requirements on documentation, reviews, and schedules.

PP-04 The point of contact for the P-PPE on all matters regarding planetary protection shall be the PPO.

PP-05 All discrepancies and unresolved critical issues on matters of planetary protection shall be immediately brought to the attention of the PPO.

C: This in particular includes any planetary protection issues with instrument providers.

PP-06 The industrial prime contractor and the launch service contractor shall make appropriate arrangement that allow the PPO to conduct verification assays on flight hardware and facilities during the course of the project.

C: This includes hardware, instrument providers with bioburden control requirements, and manufacturing sites.

PP-07 The industrial prime contractor and the launch service contractor shall make appropriate arrangement that allow the PPO to be present during the transport of the bioburden controlled spacecraft and during the launch operations.

C: This is to ensure that timely and appropriate actions are taken in case the bioburden control of the spacecraft is compromised.

PP-08 The planetary protection progress reporting shall be part of the overall project progress reporting and shall include as a minimum:
**ExoMars Project**

- Status of the planetary protection activities since the last progress report, including update of ongoing analysis, detailed assay results (with raw data), bioburden trend analysis at spacecraft and bioburden controlled cleanroom level, status of medical screening and training;
- Non-conformances and waiver/deviation status wrt planetary protection;
- CIL wrt planetary protection;
- Identified problems which may affect customer requirements, schedule, and cost;
- Planned accomplishments in the next reporting period.

**PP-09**  
All planetary protection related data and analyses shall be stored in an electronic database. This shall allow to import and export data from and to sub-contractors and the customer. The database format and content shall be agreed with the customer.

**PP-10**  
The PPO shall be notified prior to establishing contact with NASA and JPL on all matters related to planetary protection.

**PP-11**  
The final planetary protection responsibility of the mission shall reside with ESA.
6 PLANETARY PROTECTION REQUIREMENTS

6.1 General Mission Requirements

All values for bioburden limits specified in requirements are values for bioburden at launch, as per “Assay Procedure 1” described in A2.1 or estimated as per PP-39. No credit can be taken for in-flight environment or surface conditions on Mars that could reduce the bioburden (only exception that might apply is related to burn-up/break-up analysis for the CM and entry heating analysis for the heatshield – see PP-56). All values for bioburden limits specified in requirements represent maximum values and are independent of the surface area or size of the spacecraft modules.

PP-12 An organic materials inventory of bulk constituents present in quantities of 1 kg or more shall be provided for all launched hardware. The inventory shall include the following information: Identifier (e.g., FM 300 Epoxy Film adhesive), chemical composition, use (e.g., bonding of heaters for cruise stage shunt radiators), mass estimate, rating and reference for outgassing, process parameters (if processed), and supplier.

PP-13 A 50-g sample of any organic material of which 25 kg or more is used shall be archived under appropriate conditions and control for at least 50 years after launch.

PP-14 Aliquots of all spacecraft verification assays, of the bioburden controlled cleanroom(s) commissioning, and of alert and action level investigations shall be archived under appropriate conditions and control for at least 50 years after launch.

PP-15 All spacecraft hardware shall be compatible with damp swab assays as per “Assay Procedure 1” described in A2.1. 
C: Caution – see chapter on electrochemical compatibility in [NR 2].

PP-16 All spacecraft hardware shall be compatible with alcohol cleaning (IPA or ethanol).
C: Caution – specific attention has to be paid regarding compatibility with coatings, surface finishes, and TPS.
C: Caution – see chapter on electrochemical compatibility in [NR 2].

PP-17 All spacecraft hardware selected for DHMR shall be compatible with DHMR as per procedure described in A3.

PP-18 Independent of the bioburden allocation and control approach, the following spacecraft hardware shall be compatible with DHMR as per procedure described in A3:

- Harness;
- Thermal insulation;
- Solar array assembly;
- Multi layer insulation;
- Launcher fairing acoustic isolation assembly;
- Heatshield (backshield and frontshield assembly), including external and internal TPS and support structure;
• Parachute assembly;
• Airbag assembly.

PP-19 Flight spares for all spacecraft hardware that is bioburden controlled shall be treated and controlled the same way as the spacecraft hardware.

PP-20 A bioburden reserve of $1 \times 10^5$ bacterial spores for exposed surfaces shall be kept at the discretion of the PPO until launch.

C: This bioburden reserve is foreseen in case of a late contamination event. All bioburden limits in the requirements of this document are limits taking the bioburden reserve into account.

PP-21 The probability for an accidental impact on Mars by the spacecraft shall not exceed $10^{-2}$.

C: This includes all mission phases from launch until landing, including cruise, and orbital insertion.

PP-22 In case PP-21 cannot be guaranteed, the entire spacecraft shall have a total bioburden of less than $4 \times 10^5$ bacterial spores.

PP-23 Final landing site selection shall be subject to approval by the PPO.

C: This requirement is specifically to evaluate the compliance related to special regions on Mars – the ExoMars mission has to avoid special regions in its 3-sigma landing ellipse.

### 6.2 Requirements for the Launch Vehicle

PP-24 The probability of an impact on Mars by any part of the launch vehicle shall not exceed $10^{-4}$.

PP-25 The innermost part of the launcher fairing acoustic insulation assembly (exposed to the spacecraft) shall be compatible with alcohol cleaning (IPA or ethanol).

PP-26 The innermost part of the launcher fairing acoustic insulation assembly (exposed to the spacecraft) shall be compatible with damp assays as per A2.1.

PP-27 The innermost part of the launcher fairing acoustic insulation assembly (exposed to the spacecraft) shall be compatible with DHMR as per A3.

PP-28 If not stated otherwise, the launch operation after bioshield closure of the DMC shall provide uninterrupted ISO level 8 cleanroom conditions during storage, transport, launch stack assembly, fairing closure and fairing environment until launch. Appropriate contingency procedures shall be provided.

### 6.3 Requirements for the Carrier Module

PP-29 The Carrier Module (CM) shall be assembled and maintained in ISO level 8 cleanrooms as per [NR 3], or better, with appropriate controls and procedures as per [NR 4].
C: This is independent of the CM break-up/burn-up analysis but used to control the recontamination of the DM during launch.

PP-30 For bioburden control, the CM shall be considered part of the landed system with planned hard landing. Total bioburden limit applies.
C: See PP-34.

6.4 Requirements for the Landed System

The landed system includes all landed elements and separable equipment such as DM, RM, parachute, aeroshell, balance weights, and CM in a single landing event. A single landing event is one when the predicted landing location of the modules and equipment cannot be shown pre-launch to be disparate at the 3-sigma level.

PP-31 A complete organic materials inventory, shall be provided at DMC-level. The inventory shall include the following information: Identifier (e.g., FM 300 Epoxy Film adhesive), chemical composition, use (e.g., bonding of heaters for cruise stage shunt radiators), mass estimate, rating and reference for outgassing, process parameters (if processed), and supplier.
C: This requirement is more stringent than the organic materials inventory requirement for all launched hardware in PP-12.

PP-32 A 50-g (or equivalent) sample of any organic material used at RM-level, including the drill system, the SPDS, and payload, shall be archived under appropriate conditions and control for at least 50 years after launch.
C: This requirement is more stringent than the organic material archiving requirement for all launched hardware in PP-13.

PP-33 The landed system, including payload, shall be assembled and maintained in ISO level 8 cleanrooms as per [NR 3], or better, with appropriate controls and procedures as per [NR 4].
C: More stringent requirements apply for bioburden controlled operations.

PP-34 The mated and encapsulated bioburden of the landed system making a planned hard landing shall be less than 2x10^5 bacterial spores.
C: Landed system elements making a planned hard landing are for example balance weights, heatshield, and CM (see also PP-30 for CM).

PP-35 The bioburden of the landed system (the sum of soft and hard landed elements) shall be less than 2x10^5 bacterial spores on exposed internal and external surfaces. The average bioburden shall be less than 300 bacterial spores/m² on exposed internal and external surfaces.

AND

The subsystems which are involved in the acquisition, delivery, and analysis of samples used for life-detection shall have a bioburden of less than 30 bacterial spores (i.e. sterile) - or at a level driven by the nature and sensitivity of the particular life-detection experiments, whichever are more stringent - on exposed internal and external surfaces, and a method of preventing (re)contamination of the these subsystems and the material to be analyzed for life-detection shall be in place.
PP-36 If an off-nominal condition (such as hard landing) would cause a high probability of inadvertent contamination of a special region, the bioburden of the entire landed system shall be less than 30 bacterial spores on exposed internal and external surfaces.

PP-37 An analysis of spacecraft induced special regions shall be provided.

C: Spacecraft in that context means all spacecraft elements. A spacecraft can induce a special region even in a non-special region mostly by providing a long-term heat source in the presence of ground ice. This is typically the case for nuclear powered systems and applies to nominal and off-nominal conditions. As a practical consideration, the spacecraft induced effect is insignificant if the temperature is raised to less than +5°C for less than one sol per martian year (potentially excluding temperature deviation during sample acquisition). Landing site restrictions might apply based on the analysis provided.

PP-38 All parts that come in direct or indirect contact with the samples shall satisfy the cleanliness level specified.

C: The cleanliness level in this context is focused on contamination of relevance for the life-detection experiments. The maximum amount of terrestrial contamination transferred to the organic instruments per gram of sample can be considered on the order of nano-gram TOC until further clarifications. The appropriate cleanliness level for all parts that come in direct or indirect contact with the samples (e.g., drill, SPDS) to meet this requirements needs to be evaluated. See also PP-35.
7 METHODS AND PROCEDURES

7.1 Bioburden Level Estimate

PP-39 Estimation of the spacecraft bioburden shall be performed using the following values [IR 2], or preferably monitored as per “Assay Procedure 1” described in A2.1, see 7.2:

- **Average encapsulated spores density:**
  - Non-metallic portions of the spacecraft: 130 spores/cm$^3$

- **Source specific encapsulated spore density:**
  - Electronic piece parts: 3-150 spores/cm$^3$
  - Other non-metallic materials: 1-30 spores/cm$^3$

- **Source specific enclosed surface spore density:**
  - Clean room-highly controlled: 500-5000 spores/m$^2$
  - Clean room-normal control: 5000-10$^5$ spores/m$^2$
  - Uncontrolled manufacturing: 10$^4$-10$^6$ spores/m$^2$

Highest number must be used in the absence of microbial assay or sterilization processing history data.

- **Average surface spore density (exposed and mated but non-encapsulated):**
  - ISO class 7 cleanroom or better, highly controlled: 50 spores/m$^2$
  - ISO class 7 cleanroom or better, normally controlled: 500 spores/m$^2$
  - ISO class 8 cleanroom, highly controlled: 1 000 spores/m$^2$
  - ISO class 8 cleanroom, normally controlled: 10 000 spores/m$^2$
  - Uncontrolled manufacturing: 10$^5$ spores/m$^2$

These data are only applicable for bacterial spores estimation, and not for the definition of a terminal sterilization process. For estimating surface densities for vegetative microorganisms, the above values shall be multiplied by a factor of 10.

*C: Direct assays are always preferred compared to estimates.*

7.2 Bioburden Assessment Procedures

PP-40 Bioburden assessment on spacecraft hardware shall be performed as per “Assay Procedure 1” described in A2.1.

*C: Alternatively, the NASA procedure as per IR 4 can be used.*

*C: Caution – For bioburden control on spacecraft hardware, “Assay Procedure 1 (aerobic mesophilic bacterial spores)” is used; for bioburden control of cleanrooms “Assay Procedure 2 (aerobic mesophilic bacteria)” is used. See also A4.*
PP-41 Bioburden assessments on spacecraft hardware shall be performed as required to meet the bioburden control plan for flight systems (see 8.3), but at least:

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

PP-42 The number of samples required to estimate the bioburden for the flight system (i.e. last physical access) shall be based on the following guidelines:

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

PP-43 Biodiversity assessment on spacecraft hardware shall be performed as per assay procedures described in A2.5-A2.9.

PP-44 Biodiversity assessments on spacecraft hardware shall be performed as required to meet the bioburden control plan for flight systems (see 8.3), but at least:

- At the different bioburden controlled environments for the major sub-systems (e.g., integration sites for PPL/SPDS, RM, and DM, parachute manufacturing, airbag manufacturing, test site, and launch site);
- After alert and action level deviations at cleanroom level;
- Verification assays prior to launch.

PP-45 Only swabs and wipes shall be used for assays on spacecraft hardware.

PP-46 Flocked Nylon swabs shall be used for swab assays. Cotton swabs or swabs with wooden applicators shall not be used.

PP-47 Polyester wipes with sealed edges shall be used for wipe assays.

C: Caution – wipes have to be compatible with appropriate cleanroom level.

PP-48 Any use of other assay procedure is subject to approval by the PPO.
C: Alternative methods require demonstration of effectiveness by the Prime.

PP-49 All surfaces that are not accessible after delivery shall be assayed prior to delivery and the results shall be reported to the P-PPE and the PPO prior to delivery for review.

C: Verification assays of such surfaces might be taken at the discretion of the PPO. See also PP-06.

PP-50 Calculation of surface bioburden density and number of spores from a single assay shall be based on the following guidelines:

- Spore counts must be corrected upward to account for fraction plated;
- Spore counts can be pooled (including swabs and wipes) to obtain the spore number density for a single assay;
- Effective area sampled: \( A_s = n_s \times a_s \times f_s + n_w \times a_w \times f_w \);
- For spore counts of greater than one: \( \sigma_B = \frac{N^{1/2}}{A_s}; B = \frac{N}{A_s}; B_{\text{max}} = B + 3 \sigma_B; \)
- For spore counts of zero or one: \( \sigma_B = \frac{1}{A_s}; B = \frac{N}{A_s}; B_{\text{max}} = B + 3 \sigma_B; \)

C: \( N \): number of spores; \( n_s \): number of swabs; \( n_w \): number of wipes; \( a_s \): sample area for swabs; \( a_w \): sample area for wipes; \( f_s \): pour fraction for swabs; \( f_w \): pour fraction for wipes; \( A_s \): effective area sampled in a single assay of \( n_s \) swabs and \( n_w \) wipes; \( B \): spore density; \( \sigma_B \): standard deviation.

C: The numbers of samples taken at one stage of the spacecraft hardware assembly process constitute a "single assay".

7.3 Bioburden Reduction Procedures

Going through a hardware bioburden reduction procedure involves usually the following steps: analysis to select the most appropriate method, validation at breadboard and material level to identify any incompatibilities and to identify secondary effects like residues and outgassing, procedure development for applying selected method (including conditioning, packaging, functional tests, etc.), qualification of selected procedure, and finally use on flight model (and spare, if appropriate).

PP-51 Each bioburden reduction procedure shall be monitored for its ability to control the bioburden.

PP-52 Bioburden prior to bioburden reduction shall be established either by assay as per “Assay Procedure 1” described in A2.1, or by the maximum bioburden numbers per cleanroom level given in PP-39.

PP-53 The bioburden reduction procedure for spacecraft hardware shall be DHMR as per procedure described in A3.

C: Caution – hardware exposed to high temperature might produce contamination that can interfere with molecular contamination control requirements.

C: Caution – specific attention has to be paid to blocking talc (if used in parachute assembly), lubricants, coatings, finishes, adhesives, and adhesive tapes.

C: Caution – specific attention has to be paid to the extent different hardware can be sterilized in one cycle (e.g., cross contamination through outgassing, different conditioning cycle for humidity control, etc.).

PP-54 For DHMR, mated D-values shall be used for surface and mated bioburden reduction; encapsulated D-value should be used for encapsulated bioburden reduction.

C: See A3.
PP-55  Any use of other bioburden reduction procedures is subject to approval by the PPO.

C: Alternative methods require demonstration of effectiveness by the Prime.

PP-56  Systems for which it is demonstrated that a minimum temperature of 500°C is reached for at least half a second during atmospheric entry on Mars can be considered sterile [IR 2].

C: This requirement can potentially be used for heatshield, and CM. In such a case, request for waiving the “bioburden at launch” requirement is necessary and subject to approval by the PPO (see also PP-67 and PP-68).
8 PLANETARY PROTECTION DOCUMENTATION AND REVIEWS

Planetary protection reviews may be incorporated in broader project reviews. The scope of planetary protection reviews is defined by the description of the required documentation. A summary of planetary protection reviews and deliverable documentation at each milestone is given in A1. If not stated otherwise, all documentation has to be delivered 30 days prior to a scheduled review.

8.1 Planetary Protection Plan

PP-57 The Planetary Protection Plan shall be the primary planning document describing how the project will be conducted so as to meet the planetary protection requirements specified in this document.

PP-58 The Planetary Protection Plan shall include, but is not limited to, the following items:

A. General
   1. Mission description
      a. Scientific objectives
      b. Mission type
      c. Identification of targeted and/or encountered solar system bodies
      d. Identification of the use of perennial heat sources
   2. Planetary protection constraints
      a. Designation of planetary protection category
      b. Category specific planetary protection specifications
   3. General approach to planetary protection compliance, including if applicable
      a. Probability of impact for upper stage, and CM
      b. Contamination control for bioburden and general contamination as relevant to the life-detection experiments, including storage conditions for organic material

B. Planetary protection management and organisation
   1. Organisation description
   2. Responsibilities and relationships
   3. System Interface management
   4. Contractor management
   5. Data management

C. This chapter shall specifically include a description of the launcher interface, in all appropriate items listed.

C. Documentation
   1. Identification of references and applicable documents
   2. Identification of planetary protection documents to be produced

D. Facilities
   1. Identification and description of controlled facilities
   2. Activities performed
3. Hardware affected

E. Schedules

1. Identification of project milestones
2. Schedule for planetary protection documents to be produced

PP-59 The Planetary Protection Plan shall identify potential ITAR and IPR issues specifically related to materials and processes for delivered hardware with respect to bioburden and molecular contamination control.

PP-60 The Planetary Protection Plan shall be released for the PDR.

8.2 Planetary Protection Implementation Requirements

PP-61 The Planetary Protection Implementation Requirements document shall be the basic document to describe the planetary protection requirements applicable as relevant to all sub-contractors, instrument providers, the launch service provider, and manufacturing.

PP-62 The Planetary Protection Implementation Requirements document shall include, but not be limited to, the following items:

1. Bioburden allocation
2. Contamination allocation as relevant for the life-detection experiments
3. Requirements for bioburden controlled environments
4. Requirements for sub-contractors
5. Requirements for instrument providers
6. Requirements for the launch service provider
7. Requirements for manufacturing
8. Delivery and acceptance requirements

PP-63 The requirements in the Planetary Protection Implementation Requirements document shall be compliant with this document (EXM-MS-PL-ESA-00005). In case of conflict, the Prime shall inform the PPO.

PP-64 The Planetary Protection Implementation Requirements document shall be released for the CDR. A draft version with sufficient detail to issue dedicated contracts for manufacturing, sub-contractors, instrument providers, and the launch service provider shall be part of the phase B2/C/D/E1 proposal.

8.3 Planetary Protection Implementation Plan

PP-65 The Planetary Protection Implementation Plan shall be the basic document describing the detailed implementation of the planetary protection requirements, including bioburden and general contamination control as relevant to the life-detection experiments.

PP-66 The Planetary Protection Implementation Plan shall include, but not be limited to, the following items:
A. General
B. Flight system description
   1. Hardware description
      a. System and sub-system description, including instruments
      b. Planetary protection description vs. subsystem names
   2. Criteria for exposed surfaces and planetary protection accountable volumes
   3. Mission planetary protection issues
      a. Spacecraft induced special regions
      b. Landing site selection
      c. Organic material list
C. Facilities
   1. Formal system
      a. Risk assessment
      b. Alert and action levels
      c. Control approach
         • Cleanroom(s) discipline
         • Personnel controls
         • Training programme
      d. Monitoring approach
         • Particulates
         • Molecular
         • Environmental parameters
         • Bioburden
         • Biodiversity
   2. Commissioning
   3. Operation
D. Contamination control plan for the flight system
   1. Contamination source analysis
   2. Contamination control approach
   3. Contamination monitoring approach
   4. CM break-up/burn-up analysis
   5. Aeroshell entry heating analysis
   6. DHMR compatibility report
E. Bioburden control plan for the flight system
   1. Bioburden allocation
      a. Exposed surface bioburden allocation
      b. Total bioburden allocation for hard landed systems
      c. Hardware exceptions
d. Bioburden allocation accounting

2. Sampling/Assay plan
   a. Fraction of exposed surfaces sampled
   b. Number of samples required
   c. Sampling site selection
   d. Sampling schedule

3. Statistical treatment of the assay results
   a. Case for total spore count greater than one
   b. Case for total spore count of zero or one
   c. Case for treatment of bulk assay
   d. Basis for bioburden density standard deviation
   e. Assay results acceptance guidelines

4. Bioburden estimation
   a. Calculation of surface bioburden density and number of spores from assay data
   b. Surface bioburden density and number of spores without assay data
   c. Surface bioburden density and number of spores for hardware treated by a bioburden reduction process
   d. Bulk bioburden density and number of spores with assay data
   e. Bulk bioburden density and number of spores without assay data

5. Biodiversity estimation

F. Bioburden reduction plan for the flight system

1. Spacecraft hardware subject to bioburden reduction processes
   a. Identification
   b. Exceptions

2. Process analysis
   a. Analytical techniques
   b. Assumptions
   c. Process parameters
   d. Process modification

3. Process verification and control
   a. Process description and boundaries
   b. Process qualification
   c. Equipment and facilities qualification
   d. Acceptance criteria
   e. Process interruption and modification
   f. Quality assurance provisions

4. Maintaining reduced bioburden level
   a. Monitoring/assaying
   b. Using bio-barriers
c. Controlling macro-organisms (insect, animal, etc.)
   d. Contingency planning

G. General implementation approach for the flight system

1. Pre-AIV/AIT & launch operations
   a. General approach at hardware manufacturing sites
   b. General approach at Prime/subcontractor/instrument provider site

2. AIV/AIT & launch operations
   a. Delivery acceptance
   b. General approach at Prime/subcontractor/instrument provider site
   c. General approach at test site
   d. General approach at launch site

3. Inside surface of the launch vehicle fairing, launch vehicle air conditioning, and white room air conditioning

4. Upper stage and propulsion module

5. Carrier module
   a. Structure
   b. Electronics
   c. Harness
   d. Solar arrays
   e. Antennas
   f. Sensors
   g. Multi layer insulation and micrometeoroid protection
   h. Thermal control
      • Heaters and thermistors
      • Surface finishes
      • Radiators
      • Thermal insulation

6. Descent Module/Lander
   a. DM and SES Structure
   b. Heatshield assembly
   c. Backshield assembly
   d. Parachute assembly
   e. RCS
   f. Airbags assembly
   g. Electronics
   h. Harness
   i. Solar arrays
   j. Batteries
   k. Antennas
ExoMars Project

l. Radar altimeter
m. RHUs
n. Thermal control
   • Heaters and thermistors
   • Surface finishes
   • Radiators
   • Thermal insulation
o. Multi layer insulation
p. DM mounted rover equipment
q. DM mounted instruments
r. Separation and deployment mechanisms, including pyros

7. Rover module
   a. Structure
   b. Payload inside the Pasteur/SPDS biobarrier
   c. Payload outside the Pasteur/SPDS biobarrier
d. Drill and SPDS
e. Electronics
f. Harness
g. Solar arrays
h. Batteries
i. Antennas
j. RHUs
k. Thermal control
   • Heaters and thermistors
   • Surface finishes
   • Radiators
   • Thermal insulation
l. Multi layer insulation

8. Protection against recontamination
   a. Hardware pre-AIV/AIT & launch operations
   b. Hardware delivered to AIV/AIT & launch operations
c. During AIV/AIT & launch operations assembly
d. During tests
e. During storage and shipping
f. During AIV/AIT at launch site
g. Handling and storage of drapes and covers
h. Handling, storage, and use of tapes
i. Staging area for problem hardware
j. Biobarriers (flight and temporary)

H. Quality assurance

1. Pre-AIV/AIT & launch operations

2. AIV/AIT & launch operations

C: Input for this document from “End-of-Life Cleanliness Analysis” [NR 2].

C: Input for this document from “Hardware Compatibility with Bioburden Assessment Procedures” of [NR 2].

C: Input for this document from “Data for Hardware Compatibility with Processes for Bioburden Reduction” of [NR 2].

PP-67 The Planetary Protection Implementation Plan shall include a break-up/burn-up analysis of the CM to evaluate to what extend the CM or parts of the CM can be sterilized during atmospheric entry on Mars (see PP-56). Analysis shall have sufficient level of detail and confidence at element PDR to decide if the CM requires bioburden control beyond ISO level 8 environment.

PP-68 The Planetary Protection Implementation Plan shall include an entry heating analysis for the back shell and heat shield to evaluate to what extent the back shell and heat shield can be sterilized during atmospheric entry on Mars (see PP-56). Analysis shall have sufficient level of detail and confidence at element PDR to decide if a dedicated biobarrier is required for the DM.

PP-69 The Planetary Protection Implementation Plan shall identify areas of exposed internal and external surfaces on all spacecraft elements (soft landed system and hard landed system), including bioburden allocation with maximum allowable bioburden density at launch, maximum allowable bioburden at launch, and 3-sigma estimated bioburden. Allocation shall have sufficient level of detail and confidence at element PDR to issue requirements for all bioburden controlled hardware.

PP-70 The Planetary Protection Implementation Plan shall identify the accountable volume for hard landed systems, including bioburden allocation with maximum allowable bioburden density at launch, maximum allowable bioburden at launch, and 3-sigma estimated bioburden. Allocation shall have sufficient level of detail and confidence at element PDR to issue requirements for all bioburden controlled hardware.

PP-71 The Planetary Protection Implementation Plan shall include analysis and test results of all hardware elements identified for mandatory DHMR. Analysis and test results shall be released as part of a “DHMR Compatibility Report” no later than element CDR.

C: See also PP-18.

C: Input for this document from “Data for Hardware Compatibility with Processes for Bioburden Reduction” of [NR 2].

PP-72 The Planetary Protection Implementation Plan shall include an estimate on the probable recontamination during AIV/AIT and launch operations.

PP-73 The Planetary Protection Implementation Plan shall include specifications and requirements for all bioburden controlled environments used in the project (including hardware, instrument provider and launch site). These specifications and requirements shall include cleanroom monitoring for particulates, molecules, standard environmental conditions (e.g., temperature, humidity, pressure differential), bioburden and biodiversity, and specifications related to the qualification and commissioning phase. The specification and requirements shall be released no later than PDR and shall be detailed enough to issue dedicated contracts. Commissioning reports shall follow at appropriate time, but before use of the bioburden controlled environment for spacecraft hardware.
C: See A4 for bioburden controlled cleanroom specifications and requirements.

PP-74 The Planetary Protection Implementation Plan shall include a detailed description of the training programme for all personnel intended to work in a bioburden controlled environment. The training programme shall be finalized no later than 3 month after PDR and is subject to the approval by the PPO.

C: See A5 for details on the training programme.

PP-75 The Planetary Protection Implementation Plan shall include a detailed description of the medical screening programme for all personnel intended to work in a bioburden controlled environment. The medical screening programme shall be finalized no later than 3 month after PDR and is subject to the approval by the PPO.

C: See A6 for details on the medical screening programme.

PP-76 If not stated otherwise, the Planetary Protection Implementation Plan shall be released for the CDR. A preliminary Planetary Protection Implementation Plan shall be available at PDR.

8.4 Pre-Launch Planetary Protection Report

PP-77 The Pre-Launch Planetary Protection Report shall be the basic document used by the project to provide verification that planetary protection requirements have been met, and that the project can continue to satisfy them throughout the mission.

PP-78 The report shall include, but not be limited to, an update on the planetary protection compliance:

1. Orbital lifetime
2. Results of contamination control measures
3. Deviation from Planetary Protection Plan
4. Deviation from the Planetary Protection Implementation Plan
5. Organic Inventory List
6. Spacecraft induced special regions
7. Landing site selection

PP-79 The Pre-Launch Planetary Protection Plan shall be delivered 60 days before the scheduled review, and released for the FRR.

8.5 Post-Launch Planetary Protection Report

PP-80 The Post-Launch Planetary Protection Report shall be based on the Pre-Launch Planetary Protection Report, but updated to include the effects of events from the submission of the Pre-Launch Planetary Protection Report.

PP-81 The report shall include, but not be limited to, the following items:

1. Ground processing
2. Last access assay results (i.e. verification assays)
3. Launch events

PP-82 Relevant information concerning planetary protection shall be available for report to COSPAR. Report will be issued and submitted by ESA. The Prime shall provide adequate information for this report, including:

- Estimated bioburden at launch, the methods used to obtain the estimate, and the statistical uncertainty in the estimate;
- The probable composition of the bioburden;
- Methods used to control the bioburden;
- The organic inventory of all impacting or landed spacecraft or spacecraft components, for quantities exceeding 1 kg;
- Intended minimum distance from the surface of Mars for launched components, for those vehicles not intended to land on Mars (i.e. upper stage, propulsion module);
- For the end of mission, the disposition of the spacecraft and all of its major components, either in space or for landed components by position on Mars.

Information shall be submitted to ESA at the same time as the Post-Launch Planetary Protection Report, taking into account the updates provided in the Pre-Launch Planetary Protection Report and the Post-Launch Planetary Protection Report. Information shall be self-standing and not integral part of the Post-Launch Planetary Protection Report.

C: Information has to be provided in a form that allows public release.


8.6 Mission Extension Planetary Protection Report

PP-84 The Mission Extension Planetary Protection Report shall demonstrate the continued compliance with planetary protection requirements.

PP-85 The Mission Extension Planetary Protection Report shall be delivered 60 days before the scheduled review, and released for the Mission Extension Planetary Protection Review.

8.7 End of Mission Planetary Protection Report

PP-86 The End of Mission Planetary Protection Report shall provide the degree to which the project has met the planetary protection requirements throughout the complete mission and reports on disposition of all launched hardware.

PP-87 The End of Mission Planetary Protection Report shall be delivered 60 days before the scheduled review, and released for the End of Mission Planetary Protection Review, but no later than 2 month after End of Mission.
9 NON-CONFORMANCES AND WAIVERS

PP-88 Requirements in [NR 2] shall apply for all non-conformances and waivers related to planetary protection. 
C: All non-conformances affecting planetary protection requirements are classified and reported major non-conformances.
### 10 LIST OF ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIV</td>
<td>Assembly, integration, and verification</td>
</tr>
<tr>
<td>AIT</td>
<td>Assembly, integration, and testing</td>
</tr>
<tr>
<td>CDR</td>
<td>Critical Design Review</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
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<tr>
<td>CIL</td>
<td>Critical Item List</td>
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<tr>
<td>CM</td>
<td>Carrier Module</td>
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<tr>
<td>COSPAR</td>
<td>Committee On Space Research</td>
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<tr>
<td>DHMR</td>
<td>Dry Heat Microbial Reduction</td>
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<tr>
<td>DM</td>
<td>Descent Module</td>
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<tr>
<td>DMS</td>
<td>Descent Module Composite</td>
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<tr>
<td>EDLS</td>
<td>Entry, Descend, and Landing System</td>
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<tr>
<td>ESA</td>
<td>European Space Agency</td>
</tr>
<tr>
<td>ESD</td>
<td>Electrostatic Discharge</td>
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<tr>
<td>ESTAG</td>
<td>Exploration Science and Technology Advisory Group</td>
</tr>
<tr>
<td>FMECA</td>
<td>Failure Mode Effects and Critical Analysis</td>
</tr>
<tr>
<td>FRR</td>
<td>Flight Readiness Review</td>
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<tr>
<td>GSE</td>
<td>Ground Support Equipment</td>
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<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Air</td>
</tr>
<tr>
<td>HVAC</td>
<td>Heating, Ventilation, Air Conditioning, and Cooling</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol (isopropanol)</td>
</tr>
<tr>
<td>IPR</td>
<td>Intellectual Property Rights</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organisation for Standardization</td>
</tr>
<tr>
<td>ITAR</td>
<td>International Traffic in Arms Regulation</td>
</tr>
<tr>
<td>JPL</td>
<td>Jet Propulsion Laboratory</td>
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<tr>
<td>KO</td>
<td>Kick-Off</td>
</tr>
<tr>
<td>MEPAG</td>
<td>Mars Exploration Programme Advisory Group</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PDR</td>
<td>Preliminary Design Review</td>
</tr>
<tr>
<td>PPO</td>
<td>Planetary Protection Officer</td>
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<tr>
<td>P-PPE</td>
<td>Prime-Planetary Protection Manager</td>
</tr>
<tr>
<td>RCS</td>
<td>Reaction Control System</td>
</tr>
<tr>
<td>RHU</td>
<td>Radioactive Heating Unit</td>
</tr>
</tbody>
</table>
### ExoMars Project

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/C</td>
<td>Spacecraft</td>
</tr>
<tr>
<td>SPDS</td>
<td>Sample Preparation and Distribution System</td>
</tr>
<tr>
<td>SRR</td>
<td>System Requirement Review</td>
</tr>
<tr>
<td>STP</td>
<td>Standard Temperature and Pressure</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TPS</td>
<td>Thermal Protection System</td>
</tr>
</tbody>
</table>
11 TERMINOLOGY

**Action level:** Level set by the user in the context of controlled environments, which, when exceeded, requires immediate intervention, including investigation of cause, and corrective action [IR 9].

**Alert level:** Level set by the user in the context of controlled environments, giving early warning of a drift from normal conditions, which, when exceeded, should result in increased attention to the process [IR 9].

**Aseptic:** See, sterile.

**Assay:** Any activity related to gathering of microbial data through the use of appropriate sampling.

**Bacterial spores:** A resistant body produced by a vegetative bacterial cell. Capable to survive extreme environmental conditions. Generally used as reference microorganisms for the qualification of sterilisation methods. For the purpose of this document, the term *bacterial spore* describes aerobic mesophilic bacterial spores as per assay procedure A2.1.

**Bioburden:** For the purpose of this document, the term *bioburden* describes either the number of bacterial spores as per assay procedure A2.1 (for spacecraft bioburden control), or the number of bacteria as per assay procedure A2.2 in a strict quantitative sense.

**Bioburden reduction:** Operation to actively reduce bioburden. Bioburden reduction can be achieved by cleaning and/or sterilization.

**Biodiversity:** For the purpose of this document, types of microorganisms.

**Cleaning:** A process that removes contaminants (organic compounds and microorganisms) but does not necessarily sterilize the item of interest.

**Encapsulated bioburden:** Bioburden encapsulated in non-metallic spacecraft material.

**Exposed surfaces:** Internal and external surfaces whose bioburden will likely get into the martian environment from a spacecraft with no anomalies. For the use of DHMR, intent is free for gas exchange.

**Formal system:** System of contamination control with established and documented procedure [IR 10].

**Forward contamination:** For the purpose of planetary protection, contamination of solar system bodies other than the Earth by terrestrial life, including organic constituents, in the course of space missions.

**Mated surfaces:** Surfaces joined by fasteners rather than by adhesives.

**Planetary Protection:** Term used to describe the policy and the technical implementations to prevent forward and backward contamination.

**Sporicide:** A substance capable to destroy bacterial spores.

**Sterile:** State of being free from all living microorganisms (i.e. free of bioburden); in practice, usually described as a probability function.

**Sterilization:** For the purpose of this document, a process of actively reducing the bioburden.

**Total bioburden:** Total of exposed, mated, and encapsulated bioburden.
## A1 SUMMARY OF DOCUMENTATION AND REVIEWS (NORMATIVE)

PP-89 If not stated otherwise, documentation shall be available 30 days prior to scheduled review.

PP-90 Documentation shall be associated with the following scheduled reviews:

<table>
<thead>
<tr>
<th>Documentation</th>
<th>Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planetary Protection Plan</td>
<td>PDR (draft for proposal)</td>
</tr>
<tr>
<td>CM Break-Up/Burn-Up Analysis</td>
<td>Element PDR (draft at KO)</td>
</tr>
<tr>
<td>Aeroshell Entry Heating Analysis</td>
<td>Element PDR (draft at KO)</td>
</tr>
<tr>
<td>DHMR Compatibility Report</td>
<td>Element CDR (draft at PDR)</td>
</tr>
<tr>
<td>Planetary Protection Cleanroom Requirements Specifications</td>
<td>PDR (draft for proposal)</td>
</tr>
<tr>
<td>Planetary Protection Cleanroom Commissioning/Validation Report</td>
<td>Before use of cleanroom</td>
</tr>
<tr>
<td>Planetary Protection Medical Screening Programme</td>
<td>PDR</td>
</tr>
<tr>
<td>Planetary Protection Training Programme</td>
<td>PDR</td>
</tr>
<tr>
<td>Planetary Protection Implementation Requirements</td>
<td>CDR (draft for proposal, update at PDR)</td>
</tr>
<tr>
<td>Planetary Protection Implementation Plan</td>
<td>CDR (draft for proposal, update at PDR)</td>
</tr>
<tr>
<td>Organic Materials Inventory</td>
<td>CDR (draft at PDR)</td>
</tr>
<tr>
<td>S/C Induced Special Regions</td>
<td>CDR (draft at PDR)</td>
</tr>
<tr>
<td>Pre-Launch Planetary Protection Report</td>
<td>FRR</td>
</tr>
<tr>
<td>Post-Launch Planetary Protection Report</td>
<td>No later than 2 month after launch</td>
</tr>
</tbody>
</table>
A2  ASSAY PROCEDURES (NORMATIVE)

An update of the assay procedures, including contact plates, active air sampling and biodiversity assessment (4 culture assays and one PCR) for A2 will be provided at the KO. PP-104 until PP-137 are reserved for this update.

PP-91 Personnel involved with assaying spacecraft hardware shall be trained microbiologists.

PP-92 Personnel involved with assaying spacecraft hardware shall wear sterile garment consisting of a coverall, face mask, 2-layers of gloves, boots, and a hood.
   C: Caution – garment also has to be compatible with specific cleanroom level.

PP-93 Assay preparation and analysis shall be performed in a project-dedicated area of a microbiological laboratory with proper procedures to avoid cross-contamination with other users.

PP-94 Each procedure shall be validated before use, and re-validated at regular intervals [IR 5].

PP-95 The quality of prepared media shall be assessed by plating out several concentrations of the appropriate test microorganism.

A2.1 Assay Procedure 1 (Standard Assay for Swabs and Wipes)

PP-96 The Assay Procedure 1 shall be used to determine the bioburden on spacecraft hardware.
   C: With this assay aerobic mesophilic bacterial spores (and bacteria) that are able to survive a heat-shock step (80 °C, 15 min, pasteurisation) are determined.

PP-97 The area for a single sampling shall be approximately 25 cm² for swabs and 1 m² for wipes.

PP-98 The following procedure shall be followed for swabs:
   Sample collection:
   → Prepare a sufficient number of sterile swabs and test tubes with sterile grade 3 water for all swab samples to be collected, including controls.

   → Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.
   C: Moistening of swab either in a laminar flow hood (i.e. aseptically) prior to entering the bioburden controlled environment or, alternatively, inside the bioburden controlled environment just prior to swabbing.

   → Hold the swab so that the handle makes about a 30-degree angle with the surface to be sampled. While moving the swab in one direction, rotate the head of the swab slowly and thoroughly over a measured 25 cm² surface area. Change the linear direction of the swabbing motion 90 degrees and again swab the surface thoroughly. Complete a third coverage of the surface by again changing the direction of the swabbing motion by 45 degrees.
C: Templates defining a 25 cm² surface may be used.

→ Place the swab head in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint. Close the tube.

Transport and storage:

→ Transport and store samples at 4 - 8°C. Process samples within 24 hours.

Extraction:

→ Place each tube containing the buffer and the swab on a vortex mixer and vortex at maximum power for 5 - 6 seconds.

Heat shock:

→ Place the tube containing the vortexed suspension and the swab in a water bath at 80 ± 2 °C for 15 minutes, as determined by a pilot tube containing a thermometer. Make certain the water bath level is above the level of the liquid content of each tube being heated.

C: Caution – water bath temperature can fall too much if a large number of tubes are inserted at the same time. Limit the number of tubes per cycle.

→ After heat shock, cool the tubes rapidly on ice to bring the contents to 30 – 35 °C. If the entire plating procedure requires more than 10 minutes, the heat shocked tubes shall be placed in an ice bath for no longer than 45 minutes prior to plating.

Plating:

Perform plating in a laminar flow hood.

→ Vortex tube containing the buffer and the swab for 5 – 6 s.

→ Aseptically pipette 4 x 0.5 ml aliquots of the swab extraction suspension onto the surface of four R2A Petri plates, using 2 ml total.

→ Use a single-use sterile spreader to spread the dilution over the surface as evenly as possible. Allow the moisture to be absorbed into the agar before incubation.

Incubation:

→ Incubated plates inverted at 32 ± 1 °C.

Counting:

→ Examine the sample plates after 24 and 48 hours. If colonies visible by eyes are observed, count and record data. Examine and record final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72 hour count is made.

Controls:

For each ten or fewer samples collected, but at least 3 per day, collect a ‘field negative’ control.
→ Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.

→ Wave the swab through the air for 2 to 4 seconds.

→ Place the swab in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint.

In the lab, create at least two ‘lab negative controls’.
→ Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.

→ Place the swab in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint.

The quality of prepared media shall be assessed by plating out several concentrations of *Bacillus atrophaeus* spores, DSM 675, ATCC 9372.

Analyse the controls in the same way as the samples (from transport and storage to counting)!

**Equipment, reagents and consumables:**

- Dry, sterile, flocked Nylon swabs
- Tubes with sterile grade 3 water for moistening of swabs
- Tubes with 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2)
- Refrigerator (4 °C)
- Vortex mixer
- Water bath (80 ± 2 °C)
- Ice bath
- Thermometer
- Microliter pipette and tips
- Laminar flow hood
- Single-use, sterile spreaders
- R2A agar plates (90 mm)
- Incubator (32 ± 1 °C)

**PP-99** The following procedure shall be followed for wipes:

**Sample collection:**

→ Prepare a sufficient number of sterile 15 cm x 15 cm wipes pre-wetted with 3 ml grade 3 water and sterile transport tubes or jars with 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2) to accommodate all samples to be collected, including controls.

→ Rinse gloves with 70% isopropyl alcohol between each sample, and change gloves at least once every 4 samples.
→ Place the wipe flat on the sample surface and rub over the entire surface using a firm, steady pressure. Refold the wipe by reversing the direction of the open fold so the contaminated surface is interior in the new configuration. Rub the wipe over the sample area a total of three times, rotating the direction of motion 90 degrees and 135 degrees, respectively, after each complete sampling of the area. Transfer the wipe into a sterile transport jar or tube.

Transport and storage:
→ Transport and store samples at 4 - 8°C. Process samples within 24 hours.

Extraction:
Perform extraction in a laminar flow hood.
→ Add 20 ml of sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2) to each sample and reseal the jar or tube.

→ Vortex at maximum speed for 5-10 seconds, even if not very efficient for jars. Alternatively, if the wipe is in a jar that can be sealed tightly, close the lid and shake vigorously for 15 seconds.

→ Suspend sample jars or tubes in an ultrasonic bath, making sure the liquid level in the bath is above the fluid level in the sample jars or tubes and that the number of jars or tubes does not exceed the performance rating of the sonicator. Sonicate 2 minutes ± 5 seconds.

Heat shock:
→ Place the jar or tube containing the vortexed and sonicated suspension and the wipe in a water bath at 80 ± 2 °C for 15 minutes, as determined by a pilot jar or tube containing a thermometer. Make certain the water bath level is above the level of the liquid content of each jar or tube being heated.

→ After heat shock, cool the jars or tubes rapidly to bring the contents to 30 – 35 °C. If the entire plating procedure requires more than 10 minutes, the heat shocked jars or tubes shall be placed in an ice bath for no longer than 45 minutes prior to plating.

Plating:
Perform plating in a laminar flow hood.
→ If necessary, make appropriate dilutions of the wipe extraction suspension in sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

→ Vortex wipe extraction suspension for 5 – 6 s and aseptically pipette 4.0 ml portions of the suspension into individual sterile 90 mm Petri plates. A total of 32 ml of suspension should be plated.

→ Add 20 ml sterile, molten (48 - 50 °C) R2A to each plate and mix the contents by gentle swirling, and allow the mixture to solidify at room temperature.

Incubation:
→ Incubated plates inverted at 32 ± 1 °C.
Examine the sample plates after 24 and 48 hours. If colonies visible by eyes are observed, count and record data. Examine and record final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72 hour count is made.

**Controls:**
For each six or fewer samples collected, but at least 3 per day, collect a 'field negative' control.

→ Remove a sterile wipe (pre-wetted with 3 ml grade 3 water) from its transport tube.
→ Wave the wipe through the air for approximately 2 to 4 seconds.
→ Place the wipe in a sterile transport tube or jar containing 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

In the lab, create at least two ‘lab negative controls’.

→ Remove a sterile wipe from its transport tube.
→ Place the wipe in a sterile transport tube or jar containing 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

The quality of prepared media shall be assessed by plating out several concentrations of *Bacillus atrophaeus* spores, DSM 675, ATCC 9372.

Analyse the controls in the same way as the samples (from transport and storage to counting)!

**Equipment, reagents and consumables:**
- Sterile 15 cm x 15 cm wipes pre-wetted with 3 ml grade 3 water
- Transport tubes or jars with sterile buffer (20 ml PBS + 0.02 v/v % Tween 80, pH 7.2)
- Refrigerator (4 - 8°C)
- Vortex mixer
- Water bath (80 ± 2 °C)
- Ice bath
- Thermometer
- Pipette and tips
- Laminar flow hood
- Sterile spreaders
- R2A agar plates (90 mm)
- Incubator (32 ± 1 °C)
- Sterile gloves
- 70 % IPA

**A2.2 Assay Procedure 2 (Swabs and Wipes)**
ExoMars Project

PP-100  The Assay Procedure 2 shall be used to determine the bioburden in bioburden controlled environments.

*C: With this assay aerobic mesophilic bacteria are determined.

*C: Caution – do not use this assay to determine bioburden on spacecraft hardware.

PP-101  The area for a single sampling shall be approximately 25 cm² for swabs and 1 m² for wipes.

PP-102  The following procedure shall be followed for swabs:

Sample collection:

→ Prepare a sufficient number of sterile swabs and test tubes with sterile grade 3 water for all swab samples to be collected, including controls.

→ Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.

*C: Moistening of swab either in a laminar flow hood (i.e. aseptically) prior to entering the bioburden controlled environment or, alternatively, inside the bioburden controlled environment just prior to swabbing.

→ Hold the swab so that the handle makes about a 30-degree angle with the surface to be sampled. While moving the swab in one direction, rotate the head of the swab slowly and thoroughly over a measured 25 cm² surface area. Change the linear direction of the swabbing motion 90 degrees and again swab the surface thoroughly. Complete a third coverage of the surface by again changing the direction of the swabbing motion by 45 degrees.

*C: Templates defining a 25 cm² surface may be used.

→ Place the swab head in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint. Close the tube.

Transport and storage:

→ Transport and store samples at 4 - 8°C. Process samples within 24 hours.

Plating:

Perform plating in a laminar flow hood.

→ Vortex tube containing the buffer and the swab for 5 – 6 s.

→ Aseptically pipette 4 x 0.5 ml aliquots of the swab extraction suspension onto the surface of four R2A Petri plates, using 2 ml total.

→ Use a single-use sterile spreader to spread the dilution over the surface as evenly as possible. Allow the moisture to be absorbed into the agar before incubation.

Incubation:

→ Incubated plates inverted at 32 ± 1°C.

Counting:
Examine the sample plates after 24 and 48 hours. If colonies visible by eyes are observed, count and record data. Examine and record final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72 hour count is made.

Controls:
For each ten or fewer samples collected, but at least 3 per day, collect a ‘field negative’ control.

→ Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.

→ Wave the swab through the air for 2 to 4 seconds.

→ Place the swab in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint.

In the lab, create at least two ‘lab negative controls’.

→ Remove a sterile swab from its container and moisten the head of the swab in a test tube with sterile grade 3 water. Express excess moisture from the swab against the interior wall of the tube.

→ Place the swab in a tube containing 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2), and break the swab shaft at the breakpoint.

Analyse the controls in the same way as the samples (from transport and storage to counting)!

Equipment, reagents and consumables:

• Dry, sterile, flocked Nylon swabs
• Tubes with sterile grade 3 water for moistening of swabs
• Tubes with 2.5 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2)
• Refrigerator (4 °C)
• Vortex mixer
• Microliter pipette and tips
• Laminar flow hood
• Single-use, sterile spreaders
• R2A agar plates (90 mm)
• Incubator (32 ± 1 °C)

PP-103 The following procedure shall be followed for wipes:

Sample collection:

→ Prepare a sufficient number of sterile 15 cm x 15 cm wipes pre-wetted with 3 ml grade 3 water and sterile transport tubes or jars with 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2) to accommodate all samples to be collected, including controls.

→ Rinse gloves with 70% isopropyl alcohol between each sample, and change gloves at least once every 4 samples.
→ Place the wipe flat on the sample surface and rub over the entire surface using a firm, steady pressure. Refold the wipe by reversing the direction of the open fold so the contaminated surface is interior in the new configuration. Rub the wipe over the sample area a total of three times, rotating the direction of motion 90 degrees and 135 degrees, respectively, after each complete sampling of the area. Transfer the wipe into a sterile transport jar or tube.

**Transport and storage:**
→ Transport and store samples at 4 - 8°C. Process samples within 24 hours.

**Extraction:**
Perform extraction in a laminar flow hood.
→ Add 20 ml of sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2) to each sample and reseal the jar or tube.

→ Vortex at maximum speed for 5-10 seconds, even if not very efficient for jars. Alternatively, if the wipe is in a jar that can be sealed tightly, close the lid and shake vigorously for 15 seconds.

→ Suspend sample jars or tubes in an ultrasonic bath, making sure the liquid level in the bath is above the fluid level in the sample jars or tubes and that the number of jars or tubes does not exceed the performance rating of the sonicator. Sonicate 2 minutes ± 5 seconds.

**Plating:**
Perform plating in a laminar flow hood.
→ If necessary, make appropriate dilutions of the wipe extraction suspension in sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

→ Vortex wipe extraction suspension for 5 – 6 s and aseptically pipette 4.0 ml portions of the suspension into individual sterile 90 mm Petri plates. A total of 32 ml of suspension should be plated.

→ Add 20 ml sterile, molten (48 - 50 °C) R2A to each plate and mix the contents by gentle swirling, and allow the mixture to solidify at room temperature.

**Incubation:**
→ Incubated plates inverted at 32 ± 1 °C.

**Counting:**
→ Examine the sample plates after 24 and 48 hours. If colonies visible by eyes are observed, count and record data. Examine and record final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72 hour count is made.

**Controls:**
For each six or fewer samples collected, but at least 3 per day, collect a 'field negative' control.
→ Remove a sterile wipe (pre-wetted with 3 ml grade 3 water) from its transport tube.
→ Wave the wipe through the air for approximately 2 to 4 seconds.
→ Place the wipe in a sterile transport tube or jar containing 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

In the lab, create at least two ‘lab negative controls’.
→ Remove a sterile wipe from its transport tube.
→ Place the wipe in a sterile transport tube or jar containing 20 ml sterile buffer (PBS + 0.02 v/v % Tween 80, pH 7.2).

Analyse the controls in the same way as the samples (from transport and storage to counting)!

Equipment, reagents and consumables:

- Sterile 15 cm x 15 cm polyester wipes with sealed edges, pre-wetted with 3 ml grade 3 water
- Transport tubes or jars with sterile buffer (20 ml PBS + 0.02 v/v % Tween 80, pH 7.2)
- Refrigerator (4 - 8°C)
- Vortex mixer
- Water bath (80 ± 2 °C)
- Ice bath
- Thermometer
- Pipette and tips
- Laminar flow hood
- Sterile spreaders
- R2A agar plates (90 mm)
- Incubator (32 ± 1 °C)
- Sterile gloves
- 70 % IPA
A3 DRY HEAT MICROBIAL REDUCTION PROCEDURE (NORMATIVE)

PP-138 The standard dry heat microbial reduction (DHMR) process shall use the following parameters:

- Valid temperature range to receive credit for bioburden reduction: 110-125°C;
- D-value for surface and mated bioburden at 125°C: 1 hour;
- D-value for encapsulated bioburden at 125°C: 5 hours;
- z-value: 21°C;
- Upper limit for saturated water vapour partial pressure during DHMR process: 4.6 torr at STP (0°C, 760 torr);
- Calculating D-values at different temperatures in the interval [110-146°C]: \( D(t) = D(125°C) \times 10^{\frac{(125°C-T)}{21}} \);
- Calculating saturated water vapour partial pressures at different temperatures: \( P_{max} = 1.15 \times \frac{(T + 273)}{273} \);
- Using Time (t) in hours, Temperature (T) in °C, and Pressure (P) in torr.

C: Credit for DHMR at temperature above 125°C is subject to approval by the PPO.
A4 BIOBURDEN CONTROL IN CLEANROOMS

If not stated otherwise, all requirements and procedures described in chapter A4 are for bioburden controlled cleanrooms, GSE and cleanroom equipment only and NOT FOR USE ON SPACECRAFT HARDWARE.

A4.1 Formal System (Normative)

PP-139 A formal system of bioburden control shall be established, implemented, documented, and maintained within bioburden controlled cleanrooms and associated environments.

PP-140 The formal system shall include:
- Risk assessment for bioburden control (e.g., FMECA);
- Establishment of limits to ensure control (e.g., alert and action level);
- Establishment of a control scheme;
- Establishment of a monitoring scheme and schedule;
- Establishment of training procedures and access constraints;
- Establishment and maintenance of appropriate documentation.

PP-141 Action levels as per “Assay Procedure 2” in A2.2 for a bioburden controlled environment “during operations” are:
- ≥10 CFU/m³ for air samples;
- ≥2000 CFU/m² for surfaces;
- >1 CFU/glove print (5 fingers).

PP-142 Action levels as per “Assay Procedure 2” in A2.2 “during aseptic operations” are:
- >1 CFU/m³ for air samples
- >400 CFU/m² for surfaces;
- >1 CFU/glove print (5 fingers).

C: Caution – aseptic operations might require laminar air flow. This can cause undesirable electrostatic charging effects and needs to be managed.

PP-143 Appropriate alert and action levels shall be defined based on the facility commissioning phase.

C: See A4.5 for commissioning phase and A4.6 for guidelines on alert and action level.

C: Local, i.e. facility specific, action level must never exceed action level as specified in PP-141 or PP-142, as applicable.

C: General acceptance criteria are mean ± standard deviation for action levels, and mean ± standard deviation for alert level.

PP-144 Alert and action level deviations require notification of the P-PPE and the PPO.

PP-145 Alert level deviations require an investigation for equipment performance, training of personnel, trend analysis (e.g., bioburden and biodiversity), and any anomalies or discrete events. The investigation shall be completed with a deviation report.
C: Alert level deviations may or may not require corrective action.
C: Additional information on the state of the environment (e.g., particulate level) should be evaluated.

PP-146 Action level deviations require a thorough investigation and a formal non-conformance report. Investigation shall include root cause analysis, corrective action, and impact on the project.
C: See A4.6 for guidelines on elements to be covered with the investigation.

PP-147 Alert level deviations three times in a row shall be treated as action level deviation and require corrective action.

PP-148 The results of routine bioburden monitoring shall be examined periodically in order to confirm that the formal system in use is functioning in accordance with the established procedures and the specified requirements have been fulfilled.
C: This may require the development of supplementary tests and procedures, to verify that the chosen formal system is operating effectively. It could also require the systematic verification of all working steps and equipment to ensure the system is functioning properly.

PP-149 If verification indicates deviations from the established limits or a change in the microbiological status of the bioburden controlled environment, corrective action shall be initiated. If appropriate, the formal system shall be modified.

A4.2 Operational Requirements (Normative)

PP-150 Bioburden controlled environments shall be at least equivalent to cleanroom class ISO 7 “in operation” as per [NR 3], with continuous monitoring as per [NR 4].
C: Aseptic operations might require more stringent particulate control (e.g., ISO 5). Normally such conditions are provided by localized laminar air flow environments and require enclosure within at least an ISO 7 bioburden controlled environment.
C: For commissioning of bioburden controlled environment, see A4.5.

PP-151 Preparatory activities (e.g., adhesive mixing) shall be conducted in dedicated external or peripheral areas, with due regard to requirements for spacecraft bioburden requirements.

PP-152 Bioburden control shall apply to all items entering a bioburden controlled environment. This includes any (compressed) gases, liquids, general equipment, GSE, spacecraft hardware, garment, etc. A bioburden control history sheet shall accompany all items with information on the bioburden reduction and control procedures applied, the bioburden status, and the data-log to identify assay results in the planetary protection database.

PP-153 Unambiguous means to differentiate products that have been sterilized from products that have not been sterilized shall be in place.
C: Bagged sterilized items might be difficult to identify. The tracking and inventory system used should take this into account.

PP-154 Non-essential personnel shall not be permitted in the bioburden controlled environment.
PP-155 Only personnel with the appropriate level of training shall be permitted access to the bioburden controlled environment. This includes personnel for cleaning, and maintenance.

*C: See A5 for training programme.*

PP-156 Cleaning personnel employed to clean a bioburden controlled environment shall be project dedicated personnel. Regular cleaning personnel from site service are not allowed to clean in a bioburden controlled environment.

PP-157 All personnel shall undergo regular medical screening to establish that they do not have a medical condition that can compromise the integrity of the bioburden controlled environment.

*C: See A6 for examples of medical conditions of concern.*

PP-158 For routine operations on spacecraft hardware (i.e. ISO level 8 environment) personnel shall wear at least lab coat (sleeves tugged in), face mask, gloves, boots and a hat.

PP-159 For bioburden controlled operations (i.e. all operations involving bioburden controlled hardware) personnel shall wear sterile garment consisting of a coverall, face mask, 2-layers of gloves, boots, and a hood.

PP-160 Garment shall be compatible with the appropriate cleanroom standards, or better.

*C: See A4.7 for gowning procedure.*

PP-161 Sterile gloves shall be compatible with alcohol (IPA or ethanol) washes.

PP-162 Cleaning solutions used shall be sterile prior to use.

*C: See A4.7 for guidelines on cleaning solutions.*

### A4.3 Bioburden Monitoring (Normative)

PP-163 All bioburden and biodiversity assays shall be according to A2.

*C: Caution – For bioburden control on spacecraft hardware, “Assay Procedure 1 (aerobic mesophilic bacterial spores)” is used; for bioburden control of cleanrooms “Assay Procedure 2 (aerobic mesophilic bacteria)” is used.*

PP-164 The biodiversity in bioburden controlled environments shall be characterized.

PP-165 The bioburden shall be monitored with active air sampling and active surface sampling.

PP-166 For routine testing of airborne bioburden either slit sampler or filtration sampler shall be used.

*C: As per procedure in A2.*

*C: Active air sampling in change rooms during operation might give non-representative results. Passive air sampling (e.g., using settle plates) and active surface sampling in change rooms is therefore preferred.*

PP-167 For routine testing of airborne bioburden the volume of a single sample shall not be less than 1 m³.
PP-168 For routing testing of surface bioburden either swabs, wipes, or contact plates shall be used.

*C: As per procedure in A2.*

*C: Caution – do not use contact plates on flight hardware.*

PP-169 A microbiology laboratory to analyse the assays shall be in close proximity (not necessarily at the premise) to the bioburden controlled environment.

**A4.4 Sampling Plan (Normative)**

PP-170 A sampling plan shall be developed through the formal system (i.e. in the commissioning phase), documented, and adhered to.

PP-171 The sampling plan shall reflect the applied cleanliness level of the cleanroom and the appropriate degree of bioburden control required for the activity being conducted and shall include the following elements:

a) Sampling sites - the sampling locations should address the location of the primary “risk zone”; i.e. the work area immediately adjacent to areas where hardware or critical activities may take place;

b) Minimum number of samples to be taken in order to ensure representative results shall be stipulated. This number is dependant on the sample size/volume, for small sample sizes the number may need to be significantly increased in order to obtain statistically representative results;

c) Frequency of sampling – in addition to a normal, activity-level based, routine sampling frequency stipulation, the sampling plan shall define any additional samples required for critical operations or when alert levels on previous samples have been exceeded;

d) Sampling method(s), where appropriate with the number and title of standard;

e) Analysis method(s), where appropriate with the number and title of standard;

f) Factors pertinent to a particular situation that could affect results, e.g., ambient temperature, lead-time to processing of samples;

g) Impact of operations, personnel and equipment, which contribute to contamination.

PP-172 The frequencies of sampling shall be developed using the selected formal system.

*C: This system should encompass initial monitoring (i.e. during commissioning phase), routine monitoring, and contingencies for contamination events. Examples of such events which require additional sampling are:*

a) During critical activities;

b) Action levels are exceeded;

c) Alert levels are exceeded (consecutively);

d) Change in the biodiversity;

e) After prolonged shut-down of activities;

f) After any significant maintenance work has been undertaken on the cleanroom, e.g., maintenance on ventilation system, changes to furnishings or fittings, introduction of significant quantities of new hardware or equipment;

g) After changes to the process that affect the cleanroom environment;

h) After recording of unusual results;
i) After non-routine changes to the cleaning or disinfection procedures;

j) After unplanned incidents that could contribute to contamination;

k) After any incidence of non-conformance to cleanroom operational specifications, e.g.; breaches of personnel dress/conduct code, out of specification airborne particulate levels, out of specification temperature or humidity levels, exceeding maximum no. of personnel, or introduction of non-sterile items or hardware.

PP-173 Sampling sites shall be determined through the selected formal system, and included in the sampling plan.

PP-174 The location of the designated sampling sites shall be carefully selected in order to ensure all potential contamination sources are monitored – i.e. in addition to the “risk zone”, areas such as access points, storage areas with significant levels of material or hardware and significant impedance to air flow, or areas of heavy personnel traffic, shall be identified and addressed by the sampling plan.

PP-175 The labeling of each sample shall carry sufficient information or coding to provide full traceability of the sample. The following information shall be included as a minimum:

a) Collection site (facility);

b) Sampling location within facility;

c) Occupancy state at time of sampling;

d) No. of personnel present at time of sampling;

e) Date and time of collection;

f) Name of person collecting the sample;

g) Size of sample in units of m³ (volume) or m² (surface area);

h) Details of all activities being performed at the time of sampling;

i) Sampling method(s), where appropriate with the number and title of standard;

j) Analysis method(s), where appropriate with the number and title of standard;

k) Any deviations from the requirements of the sampling plan.

PP-176 Quantitative results shall be expressed as colony-forming-units (CFU) per m³ (volume) or m² (surface area).

PP-177 To assist in interpretation, results shall be reviewed over extended periods to determine trends.

A4.5 Facility Commissioning (Informative)

The commissioning phase of a bioburden controlled environment should contain the following elements:

- One detailed bioburden and biodiversity evaluation at rest;
- Five detailed bioburden and biodiversity evaluation at (simulated) operation;
- Establishing trends on bioburden and biodiversity;
- Establishing alert levels and action levels;
- Establishing a control scheme;
- Establishing of monitoring scheme.

The commissioning phase can take several months with the five detailed bioburden and biodiversity evaluation done in intervals during this period.

It is recommended to use GSE for commissioning phase. In case equipment is not available for the commissioning phase, a delta-commissioning is required.
It is recommended to use the commissioning phase to facilitate training of essential personnel.

A4.6 Action and Alert Level Deviations (Informative)

The investigation after an action or alert level deviation should be carried out in a timely manner and contain the following elements:

- Clearly identified reason for the investigation;
- Description of environment;
- Type of contaminant;
- Review sampling personnel (interviews);
- Review sampling techniques;
- Review analysis techniques;
- Review cleaning, disinfection and sterilization records;
- Review personnel monitoring, gloves and gown results;
- Review manufacturing records of items affected;
- Review overall environmental control and monitoring data of the environment, including associated environments (e.g., access areas);
- Review type of operations carried out;
- Review environment cleaning and sanitation records, including from associated environments (e.g., access areas);
- Review air pressure across the filters and inspect for any leaks in the filter;
- Check differential pressure;
- Evaluate mechanical equipment in the environment as source of contamination.

In case sampling/analysis failure has been identified as cause for the deviation, re-test results will substitute the original (faulty) test result. The original (faulty) test result should be retained for the records, with the appropriate explanation.

If no sampling/analysis error is identified in the original test, the original test results cannot be invalidated and a detailed investigation must be performed.

For inconclusive investigations where the cause of the deviation is not revealed, the out-of-specification results should be retained in the record together with the re-test analysis and give full consideration for the disposition of the item under investigation.

Atypical organisms even at numbers below the prescribed levels, should be included as triggers for corrective action.

Alert and action levels for new facilities (i.e. during commissioning) should be revisited every three to four month.

For established facilities with continuous operation (i.e. after commissioning), an annual review is sufficient.

A4.7 General Guidelines for Design and Operation (Informative)

Access to the bioburden controlled environment should be through successive layers of controlled environments, e.g., from uncontrolled environment through a standard ISO 8 environment, to a standard ISO 7 environment before entering the bioburden controlled ISO 7, environment.
It is recommended to have separate entry pathways for hardware (including GSE, spacecraft hardware, etc.) and personnel.

It is recommended to use automatic door operations to reduce cross contamination and to facilitate the controlled air movement between adjacent cleanrooms with different cleanliness class (e.g., ISO 7 and bioburden controlled ISO 7).

Changing rooms should be designed as airlocks and used to provide a physical separation of the different stages of changing. The final stage of the changing room should be at the same grade as the areas into which it leads. In general, hand washing facilities should be provided only in the first stages of the changing rooms. Alcohol dispenser to wash the hands (with and without sterile gloves) should be provided at the last stage of the cleanroom before entering the bioburden controlled environment.

Alcohol dispenser to wash the hands (with sterile gloves) should be provided inside the bioburden controlled environment.

Dispenser for sterile gloves and face masks should be provided inside the bioburden controlled environment.

Cleaning and sterilization facilities should be available in the prep-area to the bioburden controlled environment. This should include:

- Sonic cleaning;
- DHMR facility;
- Bagging capability in biobarriers, foils, and ESD for sterilization, and transport.

Sufficient storage space should be provided for sterilized tools and kits.

All exposed surfaces in the bioburden controlled environment should be smooth, impervious and unbroken.

All surfaces in the bioburden controlled environment should be compatible with alcohol cleaning (IPA or ethanol).
There should be no un-cleanable surfaces and recesses; sliding doors may be undesirable for this reason.

False ceilings should be sealed to prevent contamination from the space above them.

Pipes and ducts and other utilities should be installed so that they do not create recesses, unsealed openings and surfaces which are difficult to clean.

Back-up systems for cleanroom monitoring should be in place. Independent of the status of the cleanroom control equipment (e.g., HVAC system), specific cleanroom level can only be credited if monitored.

Special care should be taken in the fit-out at detailed level since most cleanroom suppliers are not necessarily familiar with the combined needs of facilities requiring sterility, cleanliness, and ESD.

It is recommended to provide continuous video surveillance of all critical areas in bioburden controlled environments during operations. The documented activities can be used to trace back a contamination event detected by one of the regular microbiological assessments.

In order to facilitate the identification of individual cleanroom personnel, it is recommended that all individuals in the cleanroom should be clearly identifiable by the video surveillance system (e.g., either specific symbol or colour coding of garment).

Radio communication between personnel inside the bioburden controlled environment and with personnel outside the bioburden controlled environment should be considered. This is especially recommended in laminar flow environment.

Multiple bagging should be used whenever possible to facilitate storage on-site and transport through the different cleanroom areas.

**Caution – if ESD bags are used in addition to biobarriers prior to sterilization, ensure exchange of air between the item to be sterilized and the ambient conditions in the sterilizer.**

Unpacking (removal of biobarriers), inspection, cleaning, sterilization, and related activities on GSE and flight hardware should be conducted outside the bioburden controlled environment to the maximum extent practicable (e.g., in ISO 7 prep area).

It is recommended that the flight hardware has sole occupancy of the bioburden controlled environment in all facilities. If this cannot be guaranteed, measure to prevent cross contamination have to be established and additional bioburden requirements might apply on other users of the cleanroom.

All personnel entering the bioburden controlled environment should be logged.

For planning tasks, take into consideration that it can take experienced personnel 2-4 times longer to perform mechanical or electrical assembly under bioburden controlled conditions.

Ready-to-use (i.e. sterile) garment should be available in all required sizes.
Sterile gloves with ESD finger tips should be considered.

A detailed gowning procedure should be established and implemented. This should also be part of the training. A suggested approach for entering a bioburden controlled environment is:

Prior to arrival at work:
Personnel should preferably bathe or shower the night before in order to allow natural skin oils to be re-established overnight. This practise assists in the reduction of skin particle shedding within the bioburden controlled cleanroom.

Prior to entry into the bioburden controlled facility:
Remove all personal effects (watches, rings, bracelets, any other jewellery, mobile phones, etc.) and any applied cosmetics. If applicable, remove outer garments and don intermediate garments (e.g., “scrub suits” or other undergarments, beard masks, mob caps, shoe covers etc.). Wash hands with detergent and dry with dispensable towels.

On Entry into final gowning area:
Enter room making full use of appropriate contamination control measures, e.g., air showers, tacky mats. Locate sanitising fluids/materials.

Gloves:
Select appropriate size of sterile gloves. Always handle the inner surfaces of the gloves only, i.e. inner surface of wrist of glove. If any inadvertent handling of the outer surface of the glove occurs during donning, these should be removed and discarded and a new pair selected.
Sanitise hands/gloves using IPA or ethanol and let dry.

Hood:
Select sterile hood of appropriate size. Open sterile packaging. Handle hood by inner surfaces only. Don hood in one smooth motion, taking care to ensure only inner surfaces of hood make contact with head and shoulders. If any inadvertent contact of head or body with the outer surface of the hood occurs, the hood should be removed and discarded and a new hood selected.
Once hood is fitted, check in gowning mirror to ensure hood is correctly positioned over shoulders.
Sanitise hands/gloves using IPA or ethanol and let dry.

Face-Mask:
Remove mask from sterile packaging, taking care to handle by the ties only. Position mask over hood and face, again using only the ties. Once in position, secure the mask with the ties and check the positioning in the gowning mirror. Ensure no skin is visible.
Sanitise hands/gloves using IPA or ethanol and let dry.

Coverall:
Select coverall of appropriate size. Remove coverall from sterile packaging, handling only the inner surfaces. Allow to fall to full length, taking care to avoid contacting the floor and outer surfaces of the coverall with
garments currently worn. Unzip if needed, taking care to limit the contact of gloved hand to the zip tag only. If possible, unzip from the inside of the garment.

Face coverall away from you, and gather up the arms and extra coverall, touching only the internal surfaces. Step into one leg of the coverall, avoiding contacting the coverall with the floor. Hand-rails to assist in balancing are suggested as an aid to this operation. Repeat operation with other leg, then carefully pull coverall up over shoulders, avoiding contact of any part of the body or undergarments with the outer surface. Carefully tuck hood into coverall. Zip up coverall and secure with snap-studs.

Check Coverall and hood are fitted correctly in gowning mirror.

Sanitise hands/gloves using IPA or ethanol and let dry.

**Boots:**

Select appropriate sterile over-boot size. Open sterile packaging. As per the procedure for donning gloves, care must be taken to avoid all contact with the sterile outer surfaces of the boot. Handle boots by inner surfaces only. Roll down the boot from the top, then pull boot over one foot and swing leg over boot-bar/bench into clean sector of gowning area. Leave the top of the boot rolled down. Repeat procedure with other boot. If any inadvertent handling of the outer surface of the boot occurs during donning, these should be removed and discarded and a new pair selected.

Sanitise hands/gloves using IPA or ethanol and let dry.

**Final Fitting of Over-Boots:**

Carefully roll tops of boots over legs of coverall to full extent, and secure with ties if applicable. Check entire dress is fitted in accordance with gowning procedure guidelines.

Sanitise hands/gloves using IPA or ethanol and let dry.

**Goggles/Visors:**

Select a pair of sterilised or sanitized goggles and removed from packaging. Handle goggles by straps/ties only. Position over eyes, ensuring all remaining portions of face are covered. Check fitting in gowning mirror.

Sanitise hands/gloves using IPA or ethanol and let dry.

**Second Gloves:**

Select a pair of sterile gloves of the next size up to the ones used for gowning. Remove from sterile packaging, handling by inner surfaces only. Don gloves over first gloves using same procedure as for first pair, but with the exception of ensuring that the wrists of the second pair extend over the sleeves of the coveralls, thus effecting a particulate seal over the sleeves.

Sanitise hands/gloves using IPA or ethanol and let dry.

**Entering the bioburden controlled facility:**

Perform final visual check of all aspects of dress code to ensure compliance with dress code requirements. Proceed into Cleanroom.

Always check sterile packaging to ensure it is still intact and that the items are within the prescribed expiration date. Discard any items that are past the expiration date or where the sterile packaging appears compromised.
It is a common approach to divide the bioburden controlled environment into grids. For normal bioburden control a grid size of two by two meters is appropriate. For areas with aseptic operations a grid size of one by one meter is appropriate. The details of the environment and the operations to be carried out will affect the grid size.

During the commissioning phase, one surface sample per square meter and per sampling campaign in a bioburden controlled environment is appropriate. During the routine monitoring phase one surface sample per two square meters and per sampling campaign in a bioburden controlled environment is appropriate. The details of the environment and the operations to be carried out will affect the numbers of samples per surface area.

Cleaning procedures for bioburden controlled environment should include regular cleaning of the environment with disinfectants and with sporicidals. Alternating different types of disinfectants on a periodic basis may be necessary to avoid build-up of resistant microbial population.

A system for formal scheduling and recording of all routine cleaning operations performed in the biologically controlled cleanroom should be created. This should include a Cleaning Record Sheet which identifies the areas/locations cleaned and the frequency (periodicity) of each cleaning operation (i.e. whether per shift, daily, weekly, or some other interval).

For all cleaning, disinfection, and sterilization agents used, attention must be paid to potential material incompatibilities and residues. This needs to be evaluated before use and coordinated with product assurance.

Cleaning solutions can be 2 µm-filtered to provide for sterilization.

Where disinfectants are used, more than one type should be employed.

**Caution – only use disinfectants on clean surfaces, as dust, grease or other debris can render the disinfection process ineffective.**

Cleaning sequence should generally move from the cleanest area to the less clean area, ending with the gown and de-gown rooms. It may be decided not to clean the cleanest areas at all during critical operations as cleaning is in itself a potential source of contamination.

A central vacuum system with external exhaust is preferred to HEPA filtered units with internal exhaust.

Cleaning should only be conducted during times of no work on spacecraft hardware.

**Spacecraft hardware should be covered during cleaning and maintenance.**

Doors should not be left or wedged open. Doors to another area should not be opened whilst the door to the preceding (less clean) area is still open.

Care should also be exercised in the uses of uncontrolled items by cleaning staff. All materials and equipments used for surface cleaning (mops, wipes, tacky rollers, detergents/surfactants, vacuum cleaners) should be dedicated and approved items.

Movements within the cleanroom should be slow and controlled. Erratic and rapid movements generate more particulate contamination and disturb the optimum airflow.
Good housekeeping is imperative. Areas where items are closely positioned or cluttered act as contamination traps and can result in stagnant air pockets which are not removed by the normal unimpeded airflow.

Care should be taken in positioning of personnel with respect to the contamination-critical product or hardware. Ideally, personnel should be positioned down-stream of the air flow past the product. Leaning over the product increases the risk of particulate, organic and microbial contamination. Talking whilst facing or leaning over the hardware is also inadvisable, even when wearing full face masks.

Before carrying out any operation in the cleanroom, the activity(s) should be thought through and pre-planned in order to minimise contamination.

When hand-carrying items in the clean room, these should not be held tight against the cleanroom garments, e.g., pulled into the stomach/chest, or under one arm. The outside of the garments are not 100% clean, and friction between the object being carried and the garment can generate particles and fibres. These and any associated bacteria can thus be transferred onto hardware or objects.

Personnel must not cough or sneeze whilst still facing critical hardware. If coughing or sneezing is unavoidable, the face must be turned away. Masks must be replaced after any such incident.

Personnel should avoid touching cleanroom surfaces unnecessarily. The recommended posture for the hands at rest is clasped in front of the body. Periodic disinfectant washing of the gloves as per that employed during the gowning procedure is recommended, particularly prior to critical handling operations, after contact with known contaminants, or after prolonged handling events.

Care should be taken to ensure items such as non-flight cabling, wiring or vacuum hoses are not trailed over critical hardware. The use of lay-flat polymer tubing with appropriate ESD control to encapsulate cable bundles is recommended where feasible.
A5 GUIDELINES FOR TRAINING (INFORMATIVE)

Training requires the development of useable procedures and training materials, coupled with documentation and record keeping of the performance elements applied, as well as a system for verification of training. Training may be conducted with the organization, or outside by an independent organisation.

Different levels of individual training may apply depending on the individual task descriptions. Potential training levels are:

- **Level 1**: Highest level of training; personnel can work without direct supervision in bioburden controlled environment on flight hardware (e.g., supervisor, AIV engineer, quality assurance engineer, hardware owner, etc.);
- **Level 2**: Personnel can only work with direct supervision in bioburden controlled environment on flight hardware (e.g., planetary protection support staff, general support staff, temporary visitors);
- **Level 3**: Personnel can only work with direct or indirect supervision (e.g., video surveillance) in bioburden controlled environment in the vicinity of protected flight hardware (e.g., maintenance and cleaning personnel).

The training should contain a general part to learn about the planetary protection policy, requirements, and microbiology, and a specific part to learn how to operate in a bioburden controlled environment. The latter should be specific to the training level and dominated by practical exercises in a representative environment, including work flow and process control, gowning, and cleanroom discipline.

Refresher training course should be provided on a regular basis.
A6  GUIDELINES FOR MEDICAL SCREENING (INFORMATIVE)

Medical screening for key personnel that will need to access a bioburden controlled environment should start early enough to evaluate the impact on personnel selection. By experience, a certain percentage from the general public is unfit for work in a bioburden controlled environment (1/60-1/200). Medical conditions of concern are chronic bacterial or fungal conditions that cause excessive shedding of skin scale.

Smokers release a higher amount of particulates in the period immediately after smoking. Limitations on smoking prior of entering a bioburden controlled environment should be considered.

Personnel who are ill should not be permitted access to the bioburden controlled environment if the medical condition results in either excessive particle generation, or in the inability to work in the appropriate garment. Special attention has to be paid for back-up crew during seasonal cycles of potential medical conditions (e.g., flu, allergies, etc.).