Biological Challenges and Test Program for Sterility Assurance for Mars Sample Return Planning

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The decision to implement Mars Sample Return will not be finalized until NASA's completion of the National Environmental Policy Act (NEPA) process. This document is being made available for information purposes only.

Agenda

- Back Planetary Protection
- Biological Assumptions
- Biological Challenge Plan
- Biological Challenge Selection
- Microbial Subject Matter Expert Community Request
- Example of Complied Indicator List
- Down Selection Trade Considerations
- Proposed Biological Challenge List
- Indicator Test Method
- Summary
- Backup
  - Abstract

Overview of Approach to Key Backward Planetary Protection Elements

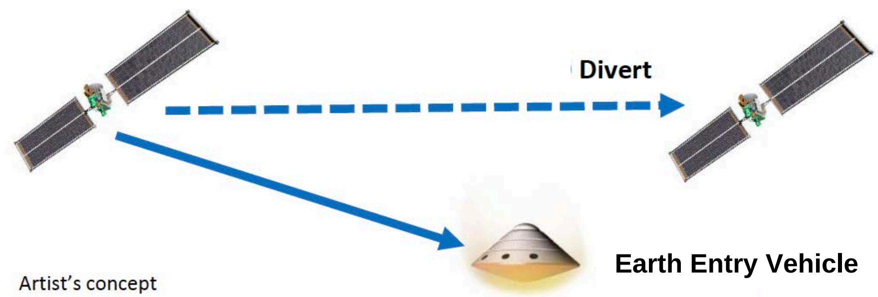
Notional Mission Phases

Mars Surface Operations	Sample Rendezvous in Mars Orbit	Earth Return Transit	Earth Arrival

Backward PP Elements

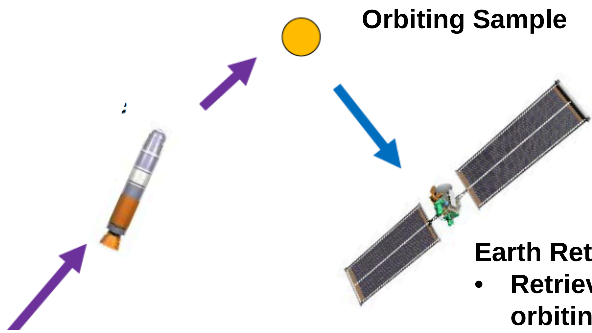
<u>Containerization, break-the-chain</u>

- Redundant robust containers
- Hardware design and/or sterilization are several options being considered



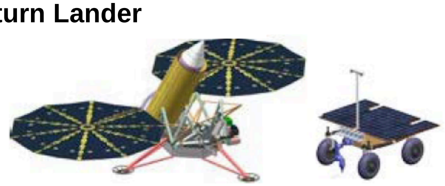
<u>Inadvertent Earth Avoidance</u>

- Orbiter off-Earth bias until shortly before sample release
- Redundant navigation and avionics systems for Earth Entry and Earth diversion of orbiter upon Earth Entry Vehicle release.



Earth Return Orbiter
- **Retrieval of orbiting sampling**
- **Break-the-chain**
- **Depart Mars**

Return Lander



Artist's concept

<u>Mars Dust and Potential Mars Biological Management</u>

- Dust avoidance hardware design and operations
- Potential use of active and/or passive sterilization measures

Back Planetary Protection

- NASA must adhere to International Policies for planetary protection by ensuring a low probability of release of unsterilized Martian particles into Earth's biosphere.
- Implementation of planetary protection on potential unsterilized Martian particles may include:
  - Containment of samples – tubes, orbiting sample break the chain, and Earth Entry vehicle.
  - Microbial reduction
    - Passive – utilizing Mars, space and Earth environments for microbial reduction (*e.g.,* desiccation, deep vacuum, UV, heat).
    - Active – evaluating potential in-flight microbial reduction systems to include (e.g., UV LED system, chemical or gas sterilization system).
- How do we ensure microbial reduction process efficacy for potential unsterilized Martian particles ?

Biological Assumptions

- Potential Mars-based biology must adhere to Earth-based physics which bounds the physical and biochemical properties (*e.g.,* carbon based chemistry) of Mars-based life.
- Forward planetary protection and terrestrial microbes leverage active replication parameters as defined by NASA NPR 8020.12 . "*Given current understanding of terrestrial organisms, Special Regions are defined as areas or volumes within which sufficient water activity AND sufficiently warm temperatures to permit replication of Earth organisms may exist. The physical parameters delineating applicable water activity and temperature thresholds are given below:*
  - *lower limit for water activity: 0.5; Upper limit: 1.0*
  - *lower limit for temperature: -25C; No Upper limit defined*
  - *timescale over which limits apply: 500 years*"

Biological Challenge Plan

Current Stage

Biological Challenge ID

- Expert Input Survey
- NASA Sterilization Working Group
- Expanded Inputs into Working Group
- Down Selection Trades
- NASA Sterilization Working Group Finalize List

Current Stage

Test Plan

- Standard Operating Protocol Development
- Test Plan Development
- Test Plan Parameter Development
- Finalize Test Plan
- NASA Sterilization Working Group Finalize Test Protocol

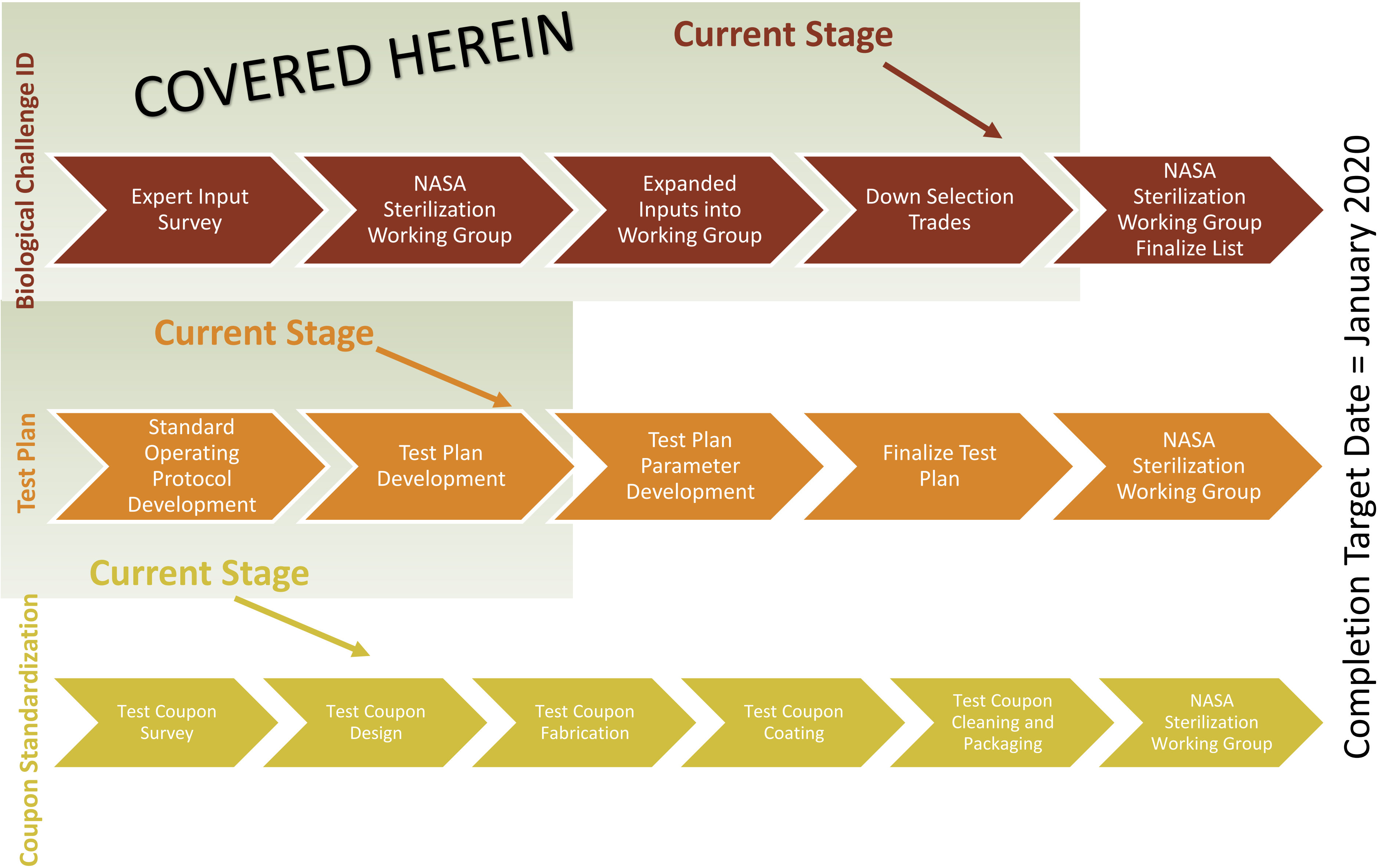
Current Stage

Coupon Standardization

- Test Coupon Survey
- Test Coupon Design
- Test Coupon Fabrication
- Test Coupon Coating
- Test Coupon Cleaning and Packaging
- NASA to inform NASA Sterilization Working Group of Coupon Approach

Completion Target Date = January 2020

Biological Challenge Plan



Biological Challenge Selection

Goal – to have the broadest engagement of the microbial community to ensure the back planetary protection sterilization.

- Identification / Exploration of biological challenge candidates
  1. Literature review and understanding of current peer-reviewed literature space
  2. Evaluation within the microbial community – polling.
  3. Expand input from External stakeholders – NASA Sterilization Working Group, other Agencies.
  4. Down selection trades – technical and programmatic

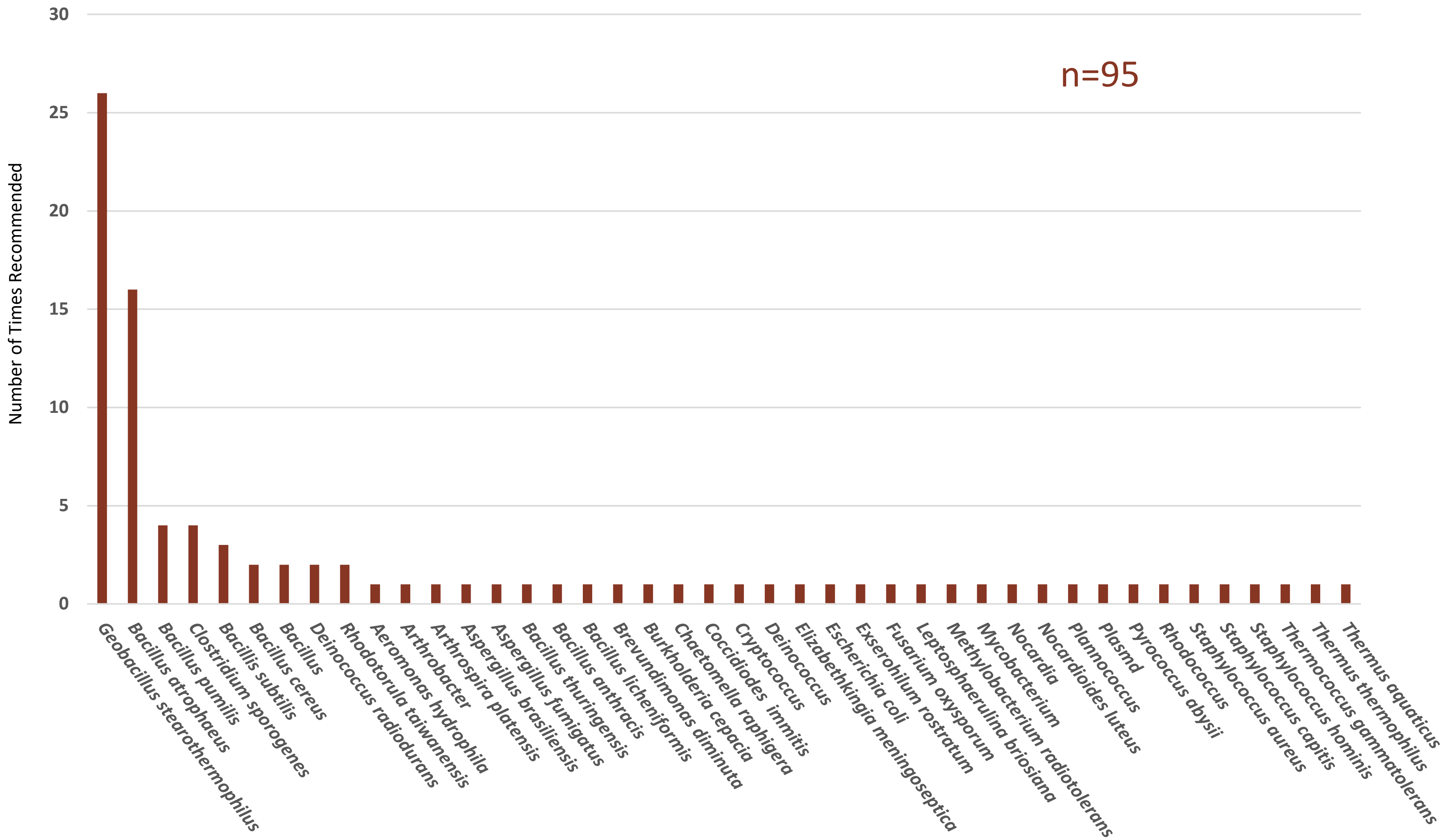
Expert Input Survey → NASA Sterilization Working Group → Expanded Inputs into Working Group → Down Selection Trades → NASA Sterilization Working Group Finalize List

Microbial Subject Matter Expert Community Request

- Question posted to subject matter experts in microbial sterilization, NASA and European Space Agency, astrobiology and environmental microbiology.
  - “ could encompass multiple domains of life (bacterial spores, viruses, proteinaceous particles, etc.). As the first step in this polling process, we ask that you:
    - Identify your…hardiest of hardies….are there hardy organisms in your lab or ones that you have come across that could be comparable to acceptable gold standards?”
- 34 labs were polled with ~50% response rate
  - 11 academic partners, 4 US Army, 4 DHS, 2 ESA, 3 FDA, 2 National Labs, 2 industry, 6 NASA, and 1 NIH potential collaborator
- Approach presented to NASA Sterilization Working Group #2 Johnson and Johnson reached out to members of the Kilmer Conference for input.
  - Added 3 more additional inputs – 2 industrial and 1 academic.

Expert Input Survey → NASA Sterilization Working Group → Expanded Inputs into Working Group → Down Selection Trades → NASA Sterilization Working Group Finalize List

Complied Subject Matter List Grouped By Species



Expert Input Survey → NASA Sterilization Working Group → **Expanded Inputs into Working Group** → Down Selection Trades → NASA Sterilization Working Group Finalize List

Down Selection Trade Considerations

- Spacecraft and space environment relevant
- Testing of industrial and regulatory biological indicators
- Testing feasibility
  – Viable but non-cultivable states
  – BSL-1 or 2 preferred
  – Specialized or customized indicators
- Resources – time and schedule

Proposed Biological Challenge List

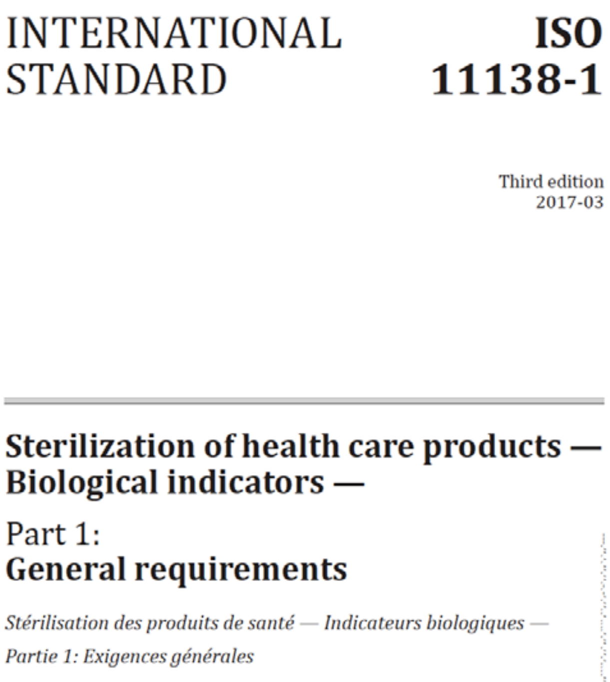
Genus	Species	Strain	Selection Rationale
Bacillus	*atrophaeus*	ATCC 9372	dry heat, ethylene oxide, steam and radiation
Bacillus	*pumilus*	SAFR-032	UV, steam
Bacillus	*sp.*	ATCC 29669	NASA heat hardy
Bacillus	*sp. E24*	DSM 30879	ESA spacecraft strain, cold plasma, H202, ClO2,
Clostridium	*sporogenes*	ATCC 11437D-5	heat resistant
Geobacillus	*stearothermophilus*	ATCC 12980	steam sterilization
Staphylococcus	*aureus*	ATCC 6538	USP 51 standard control
Brevundimonas	*diminuta*	ATCC 19146	Used for sterile filtration validation
Escherichia	*coli*	ATCC 8739	USP 51 Standard Control
Aspergillus	*fumigatus*		ISS UV resistant, more than SAFR-032 spore
Deinococcus	*radiodurans*	ATCC 35073	radiation tolerant
Rhodotorula	*taiwanensis*	MD1149	radiation tolerant yeast >66 Gy/h
Plasmd		pBR322	plasmid
Lipopolysaccharide	endotoxin		toxin; protein standard

- Additional proteins, viruses and prions are being explored and under discussion with the NASA Sterilization Working Group.
- Additional challenges may be considered to support verification program as hardware and mission design trades mature. Changes to the proposed list will be worked formally with the NASA Sterilization Working Group.

Expert Input Survey → NASA Sterilization Working Group → Expanded Inputs into Working Group → **Down Selection Trades** → NASA Sterilization Working Group Finalize List

Indicator Test Method

- What test method should be applied?
- Current Test Plan Proposed Protocol
  - ISO 11138 Sterilization of health care products – biological indicators (as relevant; particularly experimental design) as a minimum for testing
  - Coupon testing should be conducted using spacecraft materials
  - Utilization of uncontrolled / low humidity to mimic spacecraft active systems.


INTERNATIONAL STANDARD

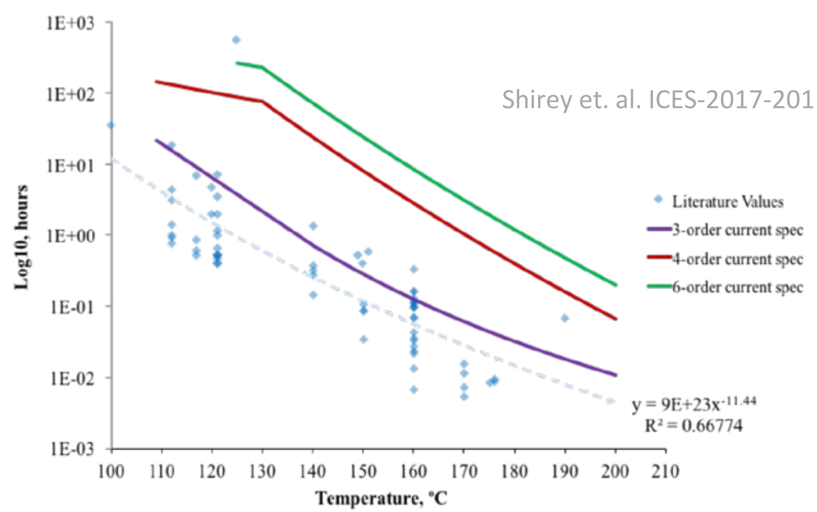
ISO 11138-1

Third edition
2017-03

Sterilization of health care products — Biological indicators —

Part 1:
General requirements

Stérilisation des produits de santé — Indicateurs biologiques — Partie 1: Exigences générales



Shirey et. al. ICES-2017-201

Developing test plans to incorporate International Standards and previous NASA testing.

Standard Operating Protocol Development → Test Plan Development → Test Plan Parameter Development → Finalize Test Plan → NASA Sterilization Working Group

Summary

- Polled the scientific literature and community to assess the current state of biological challenges.
- Proposed a biological challenge list with inputs from NASA, ESA, academic, and industrial partners to test for MSR planning.
- Evaluating and developing a test program for microbial community agreed upon standard biological challenges.
- Continued engagement with scientific community and NASA Sterilization Working Group.

Future Work

- Finalize biological challenge list with NASA Sterilization Working Group
- Develop test plan and associated parameters for high heat (125ºC – 360ºC), vapor hydrogen peroxide, chlorine dioxide and radiation.
- Design and fabrication of coupons to support testing of biological challenges on spacecraft relevant materials.
- Mars environment testing (e.g. UV, vacuum, temperature and atmosphere) on Bacillus spores initiated with preliminary studies concluding in FY20. Generating data to bolster predictive lethality models for passive space environmental conditions.
- Transition from preliminary to large scale testing, multiple facility microbial modality testing.

Questions?

Abstract

NASA's next Mars rover launching in summer 2020 will collect and cache promising samples of Mars rock and regolith on the Red Planet's surface. In parallel, the agency is studying a Mars Sample Return Campaign architecture that includes several conceptual missions that could bring those samples back to Earth someday. A fetch rover, Mars ascent vehicle, capture orbiter, Earth-entry vehicle, and sample containment facility are the notional components that would be critical to the success of such a Mars Sample Return Campaign. To prepare for sample return, NASA must adhere to International Policies for planetary protection by ensuring a low probability of release of unsterilized Martian particles into Earth's biosphere. To explore the feasibility of this requirement, based on current sterilization modalities in use, NASA has assembled a sterilization working group comprised of federal, international, industry, and academic subject matter experts. Based on available data gained from previous Mars missions, one key design assumption the working group has agreed upon for the probabilistic risk assessment is that potential Mars-based biology must adhere to Earth-based physics which bounds the physical and biochemical properties of Mars-based life. A series of sterilization/inactivation steps including passive environmental conditions (e.g. solar radiation, UV, deep space vacuum) and active spacecraft systems (e.g. chemical, heat, UV) are being considered to microbially reduce any possible initial Mars-based life starting at the launch into Mars orbit through Earth containment. To validate these active and passive processes, biological indicators (BIs) are being explored for ground-based testing to establish D- and z-values for both single and the additive impact of simultaneous modalities. NASA is taking a widely proven approach to gain expert input for selecting BIs, by surveying the scientific community for the widest spectrum of extreme environmental isolates to include bacterial, spore forming, fungal, archaeal, plasmids, and prions. Preliminary polling identified ~75 candidates, and 13 candidates were down-selected to begin initial testing. Draft test procedures will include relevant portions of the international standard experimental design defined in ISO 11138 "Sterilization of health care products - Biological indicators" and will be performed on spacecraft materials. Future work includes finalizing the candidate list of BIs, standardizing testing procedures, and establishing the candidates D and z-values as input into a probabilistic risk assessment which would inform the spacecraft design and operational scenarios.