ASEPTIC ASSEMBLY PROCEDURES FROM MARS 2020 AS A REFERENCE FOR PROTOCOLS TO PREVENT FORWARD AND BACKWARD CONTAMINATION ON HUMAN MARS MISSIONS.

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Introduction: The Mars 2020 mission's meticulous approach to biological and molecular contamination control of the rover and its component hardware [1, 2] offers valuable insights that can be adapted to the design and operation of spacesuits for future humans to Mars endeavors. Given the stringent requirements for planetary protection, spacesuits intended for human Mars missions must 1) prevent the forward contamination of Mars, ensuring that terrestrial microorganisms do not compromise Martian environments for sample science or future sample return missions and 2) prevent the backward contamination of the Earth by way of the habitat and returned astronauts or the return vehicle.

The Mars 2020 mission successfully employed hardware design [3], transport modeling [4], cleaning [1, 2], and aseptic assembly procedures [1] to achieve and maintain unprecedented levels of cleanliness. These practices can be translated into spacesuit protocols through a multi-faceted strategy that emphasizes biological contamination control at every stage—from design to operational use on Mars.

Design Considerations: In designing spacesuits, the principles of Mars 2020's hardware can be directly applied, such as research results in fluid mechanical particle barriers (FMPB) and particle transport, to better understand the dust load that could penetrate into the suit or habitat. For instance, the use of FMPBs can be adapted to spacesuit design by incorporating layers that protect against both particulate and microbial contamination. Additionally, spacesuits could be constructed with the sterilization modality in mind such that a clean surface can be maintained, akin to the assembly of the rover's sample tubes in the sheath. This approach allows for a sustainable approach to repeated sterilization of spacesuits that may have been recontaminated due to the proximal humans.

Monitoring Considerations: The aseptic assembly of hardware intended for contact with Martian rock and regolith utilized a comprehensive set of tools to monitor potential environmental and surface contamination risks. Some systems provide

rapid contamination assessments, such as ATP testing, while others, like MALDI-TOF, take a few days longer to offer a more detailed analysis. Together, these tools create a complete picture of the contamination levels, ensuring stringent protection measures are established and maintained.



During the Mars 2020 aseptic assembly of hardware, stringent facility and hardware cleanliness monitoring was conducted using witness plates (settling and contact plates) to detect potential contamination. A total of 7,619 witness plates were deployed across 688 assembly activities, with plates incubated and monitored for microbial growth.

- Settling Plates: 4,147 were deployed, mostly in ISO 5 environments, with 10 incidents leading to 19 colony-forming units (CFUs) detected. Any contamination led to cleaning and sampling, with final hardware sterilization at 150°C for 24 hours.
- Contact Plates: 3,472 were collected from assembler gloves, resulting in 23 incidents and 95 CFUs. Incidents prompted vaporized hydrogen peroxide (VHP) sterilization and subsequent baking of the hardware.

These measures ensured minimal contamination risks during assembly.

Material Selection and Sterilization:

The materials chosen for Mars human habitats and spacesuits must withstand both the harsh Martian environment and rigorous, repetitive sterilization processes. Mars 2020 demonstrated the efficacy of

Heat Microbial Reduction (HMR) and VHP sterilization techniques, which together with other sterilization methods, could be similarly employed for spacesuits. These materials must be resistant to degradation under repeated sterilization cycles, ensuring long-term reliability and cleanliness. Advanced materials that are inherently less prone to microbial adhesion could also be considered.

Operational Protocols: Operational protocols for habitat operations and spacesuit use on Mars can mirror the contamination control strategies employed during Mars 2020's sample intimate hardware assembly phases. For instance, before extravehicular activities (EVAs), spacesuits could undergo a final sterilization process similar to the "bakeout" used for hardware inside a hermetically sealed CASE (cleaning and storage enclosure) before its final deployment and integration steps. This could involve an enclosed sterilization unit accessible by humans through a port, ensuring that the vast majority of biological contaminants introduced to the spacesuit remain on its interior. Once the port is closed, the interfaces can be cleaned locally to eliminate contaminants before the suits are exposed to the Martian environment.

Additionally, just as Mars 2020 relied on constant monitoring of bioburden levels, Mars missions should employ real-time monitoring systems within the habitat and the spacesuits to detect and mitigate any contamination events. These systems could alert astronauts to potential breaches, allowing for immediate corrective actions.

Post-EVA Procedures: After EVAs, spacesuits should be carefully contained and decontaminated before being returned to storage. This would involve a containment area separated from the habitat, where suits are isolated and sterilized between uses. Drawing from the Mars 2020 protocol of using settling and contact plates to monitor contamination, space suits could be swabbed and tested to confirm their cleanliness before they are reused.

Conclusion: The Mars 2020 mission's aseptic assembly procedures provide a robust framework for developing Mars space suit protocols aimed at preventing forward and backward contamination. By integrating advanced sterilization techniques, real-time monitoring, and strict contamination control measures at every stage, Mars spacesuits can meet the high planetary protection standards required for responsible

human exploration. These protocols would not only protect the Martian environment but also ensure the integrity of scientific research conducted on the Red Planet. Continuous refinement and adaptation of these protocols would be crucial as missions advance and our understanding of Mars evolves.

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