

## PLANNING CONSIDERATIONS FOR MARS SAMPLE RETURN CONTAINMENT

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### ABSTRACT

With a Mars sample return mission planned for launch as early as 2005, serious discussions have begun to identify practical, effective approaches for containing extraterrestrial rocks, soil and atmospheric samples for return to Earth. In addition to satisfying planetary protection and biological safety requirements, containment designs must also meet planetary science and spaceflight engineering needs. To be effective, an integrated containment system must consider both outbound and inbound spaceflights, mobile and stationary containment, and operations on Earth. Important planning considerations include pre-launch preparations, the configuration and sealing of the sample return canister, Earth-return and recovery procedures, contingency planning, laboratory design, biosafety cabinets, sterilization methods, and alternative operational and facilities approaches. Solving the challenges of containment for extraterrestrial samples will be critical to mission success, not only for samples from Mars, but ultimately for sample returns from other bodies of biological interest within the solar system as well.

### INTRODUCTION

Within the next decade, the world's space agencies plan to launch a variety of robotic spacecraft that will return samples from the surface of Mars, the tail of a comet, the nucleus of a comet, the surface of an asteroid, and the solar wind. Any mission returning from a location with the potential for harboring life will require special containment and support systems. Samples from locations with no biological potential require no special containment other than that required for scientific purposes. At present, international policies require avoidance of cross contamination, although no specific guidelines are in place for sample return missions. The National Research

Council's Space Studies Board has provided useful advice and recommendations on sample return issues in two recent studies conducted at NASA's request (NRC, 1997, 1998). Drawing on these reports, scientists, engineers, and space administrators have begun serious discussions of what constitutes suitable containment for a sample return mission from Mars. The launch of a roundtrip mission is scheduled for as early as 2005 with a return to Earth around 2008. Because life on Mars is uncertain, it will be prudent to develop conservative containment designs and treat all unsterilized Martian samples as hazardous until proven otherwise. This approach will require integrating information from traditional biosafety containment facilities and support systems with details about the nature of the anticipated sample materials; scientific, technical and regulatory requirements for quarantine in containment; and lessons learned from other experiences with extraterrestrial materials.

Planning considerations must span the entire mission, from pre-launch through sample return, including equipment and operations in space and on Earth. These have implications for the ultimate effectiveness of sample isolation, containment and scientific interpretation. An integrated containment system will be required that encompasses both mobile and stationary types of containment capable of handling samples from the time they are collected *in situ* on Mars through their delivery and testing at a sample receiving facility—most likely on Earth. The integrated containment system must be designed to avoid two types of potential cross contamination—*forward contamination* of Mars by terrestrial microbes on the outbound spacecraft or equipment, and *back contamination* of Earth by returned martian materials that could contain living extraterrestrial entities. Ultimately, the design must be conservative enough to protect investigators and the biosphere from potential biological hazards, which are speculative or totally unknown. Moreover, the design must preserve the samples'

scientific value, avoid false positives, and contribute to the ultimate goal of sample return to support world-class scientific investigations.

## CONTAINMENT CONSIDERATIONS

In order to design a containment system for extraterrestrial materials, it is important to understand the nature of anticipated materials and the conflicting constraints that will challenge designers. Containment as seen by space scientists is driven by two distinctly different emphases: (1) traditional biosafety and planetary protection concerns, and (2) sample protection and science considerations. The former emphasizes keeping materials in, while the latter emphasizes keeping contaminants out. In addition to these concerns, containment designs must be suitable for the unusual conditions associated with spaceflight itself.

For the 2005 mission, returned samples will include soils, rocks, and atmospheric gases from Mars. Although the likelihood of finding biological entities in these materials is considered quite low, the probability is not zero. While previous robotic missions to Mars failed to detect evidence of life, it is possible that extraterrestrial soils, rocks and ices on the planet could serve as habitats for active or dormant microorganisms, just as they do on Earth. Based on current information, if extraterrestrial organisms are found in returned samples, they are most likely to be simple and microbial in nature.

### Planetary Protection Needs

The need for biosafety containment is driven in large part by concerns about planetary protection and avoidance of potentially harmful effects to Earth's inhabitants or biosphere that could be caused by biological entities in returned materials. The Outer Space Treaty of 1967 specifically requires that all space exploration be done in a way that avoids harmful contamination to celestial bodies or adverse changes on Earth from the introduction of extraterrestrial materials. There is no doubt that strict containment is warranted because of Mars biological potential and interest. The task is how to integrate applicable international space policies and established biocontainment guidelines (CDC-NIH, 1993, 1995) into appropriate containment facilities, equipment, laboratory designs and operational protocols for extraterrestrial materials with biological potential.

### Planetary Science Needs

Whether or not extraterrestrial samples are of biological interest, strict isolation and containment systems are also required to rigorously protect extraterrestrial materials from Earthly contamination while they are studied. The containment concerns of the space science community are driven by the need to protect the scientific integrity of returned materials, avoid false positives, and preserve samples in a pristine and unaltered state, down to the isotopic composition level. The space science community has historically done containment by means of physical barriers, such as containers and cabinets that minimize exchange of gaseous molecules, prevent exposure to terrestrial contaminants, and preserve samples in a pristine state.

### Spaceflight Engineering Needs

In addition to satisfying biosafety and planetary science concerns, additional design constraints are imposed by spaceflight engineering needs. All materials and mechanisms used during extraterrestrial sample return missions must work in the space environment of extremely low pressure, low and high temperatures, vibration, gravitational changes, and prolonged, intense radiation. Robotic containment mechanisms must be reliable and their allowance for mass and power consumption will be extremely strict because of the size and weight restrictions imposed by spaceflight. In addition, there must be a careful integration of mobile and stationary containment mechanisms and systems used in flight and on Earth.

### Balancing the Needs

There are some important philosophical differences in the technical approaches used by the biosafety and planetary science communities. Biocontainment employs negative pressure gradients in glove boxes and rooms, so that particles and gases flow inward toward samples, thereby preventing the escape of any biohazardous materials. In contrast, sample cleanliness and containment for planetary science is maintained with positive pressure gradients so that contaminant particles and gases flow away from samples and prevent the mixing of Earth contaminants with pristine samples. Thus, handling extraterrestrial samples with biological potential may require a positive pressure envelope surrounded by a negative pressure envelope to protect both Earth and sample materials.

Another potential conflict can be seen in the use of sterilizing chemicals, which along with heat and radiation, are basic components of biocontainment operations. Many of these chemicals are considered to be contaminants to extraterrestrial samples, even at extremely low levels. Curators of planetary geoscience materials strive to provide ultra clean, but not necessarily sterile, environments for sample handling operations. The materials allowed to come in contact with samples are strictly limited to materials of simple and known composition that can be easily recognized as contaminants if observed in samples. Each item going into an extraterrestrial sample cabinet undergoes precision cleaning to remove particles and films. In addition, certain elements which interfere with critical scientific measurements are not even allowed into the laboratory.

### **PREVIOUS EXPERIENCES WITH EXTRATERRESTRIAL MATERIALS**

At present, there is no single laboratory in the country that can provide the necessary combination of biocontainment, planetary science containment, and curation required for handling and testing extraterrestrial materials of unknown biological potential. In addition, there are no established standards or guidelines for collecting, containing and transporting extraterrestrial samples. Although containment, transport and handling of returned extraterrestrial materials will undoubtedly be complicated, it is not unprecedented.

Facilities for handling a variety of extraterrestrial samples have been in operation since 1969 at the Johnson Space Center (JSC) in Houston, Texas. The Lunar Receiving Laboratory (LRL), which was established during the Apollo Program, provided extensive working experience with back contamination control, quarantine, and biocontainment. Although the LRL no longer exists, experience with non-biological extraterrestrial samples has continued over the past several decades at JSC facilities through the routine handling of meteorites, lunar materials and interplanetary dust samples. Lessons learned from these facilities have contributed important design input for future extraterrestrial sample handling and processing (Allton, et al., 1998).

### **PLANNING FOR MARS SAMPLE RETURN**

As part of the planning for future Mars sample return, a Mars Sample Quarantine Protocol Workshop was held to develop preliminary guidelines for returned sample containment and quarantine analysis (DeVincenzi, et al., 1999). Participants at the workshop included representatives of government agencies, academic institutions, and aerospace industries with expertise in biosafety, research, regulations and technical areas relevant to sample return. As a result of discussions at the workshop and afterward, a number of approaches to effective containment have been outlined and debated. The following section provides an overview of the technical issues facing various phases of a Mars sample return mission and illustrates the complexity of design and operational problems that must be solved. Detailed designs for the actual containment system have not yet been determined and will require input from experts in many fields.

#### **Pre-Launch**

Contamination and containment concerns actually begin long before launch from Earth, and include avoidance of both forward and back contamination. Because of the sensitivity of analytical equipment, the spacecraft, rovers and *in situ* equipments must be cleaned thoroughly before they leave Earth to remove terrestrial contaminants that could become false positives upon return. Outbound equipment, including the sample return canister itself, must be cleaned and sterilized in such a manner that organic and microbial residues or chemical contaminants are reduced below detection limits. If such technology does not yet exist, it must be developed in time for anticipated launches.

In anticipation of analytical work at low detection limits, there will be a need to collect and preserve pre-flight portions of spacecraft materials for future comparison and use as controls in analytical procedures. These reference materials, which include sample containers and "witness" plates (controls) for particulate and biologic contamination, must be processed exactly like the flight hardware.

#### **Sample Return Canister**

On Mars, samples will be robotically placed into a sterilized sample return canister (SRC) and

sealed. The SRC will then need to be sealed inside an outer container or containers for transport back to Earth. It is unclear whether or how current regulations and packaging requirements for biological materials will apply to these extraterrestrial containers. Decontamination of the external container to break the chain of contact with the martian surface must be part of the mission design. In addition, some type of aseptic transfer to the Earth return vehicle (ERV) will be required. Overall, what is desired is "containment" of extraterrestrial materials, not necessarily conventional "sealing." Additional research and development of SRCs will be needed to evaluate alternative practical methods for achieving containment along with a contaminant-free transfer and return of sample materials.

### **Contingencies During Flight, Reentry, and Recovery**

The canister design and return procedures must also include considerations of contingencies during the return flight. A monitoring device could be used to determine if there has been a compromise of the containment en route (e.g., detection of pre-loaded inert tracer substances on exterior surfaces of collection devices by sensitive detector equipment in the return spacecraft). In the event that a containment breach is detected en route, a mission design contingency should be provided for either sample sterilization en route or diversion of the spacecraft from an Earth-reentry trajectory.

Preparations must be made to recover the intact SRC immediately following reentry. Upon confirmation that the sample has been properly contained, it will be necessary to move the SRC in an appropriate durable transport box to a quarantine facility. The manner of movement must be consistent with regulatory requirements for safe transport of potentially hazardous materials and approved by pre-arranged permits through relevant regulatory agencies to ensure a rapid and uncomplicated transfer activity.

Contingency plans will be needed for possible mishaps that could occur during recovery of the spacecraft or SRC. Such planning would likely involve multiple agencies (e.g., Environmental Protection Agency, Public Health Service, Department of Transportation, etc.) with responsibilities in emergency response. In preparation for possible accident scenarios, there

will be a need to develop methods to decontaminate the landing area in a manner that meets or exceeds regulatory requirements for the clean-up of hazardous spills. Such cleanup activities might require the use of personnel protective garments or equipment in the event of damage to the canister during reentry, landing or transport.

### **Laboratory Containment and Sample Handling**

Conditions at the sample receiving laboratory must meet the three main objectives required of all biocontainment facilities: 1) protect the laboratory workers, 2) protect the environment (in this case the entire biosphere), and 3) protect the integrity of the sample. The unknown nature of potentially hazardous material in the returned samples demands the most stringent containment presently afforded to the most hazardous biological entities known on Earth. Thus, the design will mostly likely be equivalent to BSL-4 as outlined by CDC-NIH guidelines (1993).

For economic considerations and relative simplicity, maximum biocontainment for returned extraterrestrial samples may be achieved through the use of several Class III BSCs welded together or suitably sealed into a cabinet line, connected in sequence with sealable doors between cabinets, and maintained under negative pressure. Although considered as a line, each cabinet would be capable of being isolated and operated independently and include separate supply and exhaust air with double high efficiency particulate air (HEPA) filters on both. Because martian gas samples will be collected at the time of opening the SRC, another design consideration may include possible supplemental filtration in the supply and exhaust air. The doors between the cabinets would have bioseals to ensure complete containment. Access ports (e.g., armholes with attached gloves, half-suits attached to the work surface) would be installed specifically for the unique tasks to be performed in each cabinet. Glove boxes can be designed to accommodate any laboratory equipment required by the protocols, with operational parts housed inside, and electronics, control panels, etc. located outside the primary containment barrier. Because of the need to minimize introduction of organic contaminants, special attention will be required in the selection of suitable materials for gloves, seals, and other flexible items associated with BSCs. For preservation of the science value of samples, the

selection of cover gas composition, temperature and absolute pressure must also be addressed by a science advisory group.

Secondary containment would be provided by a maximum containment structure or building, fitted with special seals, maintained under negative pressure, with HEPA filtered exhaust air, waste water sterilization, provisions for personnel showering and appropriate use of disinfectants, and other support systems. As discussed above, decontamination procedures for the interior of the cabinet line must be such that carbon-nitrogen residues or living or dead microbes are not detected. Considerable research will be needed to develop cleaning and decontamination methods that are more suitable for use with extraterrestrial materials than currently used in biomedical or industrial methods (e.g., chemical disinfectants or gaseous formaldehyde). In addition, challenge testing of HEPA filtration systems is advised prior to actual sample handling by using inert particles in the range of 10-100 nm in size.

Operational approaches for transfer and opening the SRC and handling samples inside the gloveboxes will require considerable design attention to prevent breaches in containment or sample contamination. Both the inside and outside surfaces of the incoming transport box will be contaminated with Earth organisms during the process of depositing the SRC inside. Thus preparations must be made for the complicated process of removing the SRC from the transport box, decontaminating surfaces, and movement through the sequence of cabinets. The pristine inner sample must be protected from contaminants picked up during recovery, transit and handling. The actual deployment of outer canister and martian samples through the cabinet line will depend on what tests are run at what stage of the process. Details on the exact assays and equipment required for life detection analyses and biohazard testing are still under consideration.

### **Sterilization**

Based on current recommendations, no unsterilized materials from Mars should be released from containment until rigorous analyses determine that they do not constitute a biological hazard. Moreover, "if any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized" (NRC, 1997). An acceptable method for sterilization of

subsamples must be available at the receiving and containment facility before materials are returned to Earth, regardless whether the sterilization option is exercised during actual sample handling.

A variety of techniques are routinely used to sterilize terrestrial biological samples depending on the physical characteristics of the sample and the desired results. In general, while these methods are effective for killing any viable organisms, they are not compatible with the goal of preserving the scientific integrity of extraterrestrial samples. Previous analyses of different sterilization techniques on the full range of physical and chemical properties anticipated in extraterrestrial samples indicated that no single technique will be optimum for sterilizing Mars samples (Bogard, et al., 1979; and Gooding, 1990). Dry heating and steam heating, in particular, could result in irreversible degassing and strongly alter the structure of many secondary minerals. Treatment with gas, vapor, plasma or ultraviolet light would sterilize sample surfaces but might not fully penetrate into rocks. At present, exposure to high doses of gamma radiation is being investigated as a method that may be the least damaging to rocks and soils (Allen, et al., 1998). Additional research will be needed to identify suitable sterilization methods and equipment for incorporation into a sample return facility.

### **Alternative Facility Concepts**

Clearly, biological safety and physical security must be the prime considerations in the design of any receiving and containment laboratory. The currently recommended containment approach, Class III BSCs sealed sequentially, is inherently flexible, and may be engineered to any size required for quarantine testing. However, a sample receiving facility may be established in a number of ways. Alternative suggestions to date include the construction of a new dedicated facility, limited term use of an existing biocontainment laboratory, or use of a modular, perhaps mobile quarantine laboratory. Each approach has its advantages and disadvantages.

For example, a small sample quarantine and processing laboratory could be established adjacent to an existing BSL-4 maximum containment facility, thus ensuring the availability of experienced laboratory, safety and engineering support teams from an agency or institution skilled in biocontainment. Another possibility might be to

acquire and use a number of commercial, modular biocontainment laboratories that could be shipped to an appropriate location, perhaps even the landing site itself. Such laboratories could be outfitted with the necessary equipment and technical expertise for preliminary analyses and biohazard screening. They may be equipped for initial testing behind a biobarrier, while reserving subsequent research for more fully equipped laboratories elsewhere after samples are deemed safe for release from containment. The disadvantages of using an existing BSL-4 facility include possible reduction of control of the sample by NASA and inadvertent organic contamination of samples with "carry-over" from residual laboratory materials in the existing facility. The problems associated with the use of modular, or mobile laboratories will clearly need further study.

Alternatively, the unique nature and contaminant concerns of martian samples may require construction of a dedicated containment and analysis facility, which could also be used for future sample returns from other extraterrestrial locations. In this scenario, both initial screening and all subsequent analysis would be done in one laboratory, with a core staff and provisions for visiting researchers. This solution maintains local control over samples if proven hazardous and eliminates potential problems when transporting materials between facilities. Construction, equipping and staffing of a new laboratory also has the advantage of bringing state-of-the-art equipment and techniques to bear on all aspects of sample control and analysis. However, performing all analyses at a central facility would probably prevent analysis of the samples at other world-class laboratories and limit access to valuable sample materials to a few selected science teams.

Hazard analyses for returned Martian samples will involve investigation of not only pathogenicity, but also ecological effects that could be caused by possible organisms of unknown characteristics. It has not yet been determined what equipment and instruments will be needed for these analyses, or how to accomplish subsample sterilization as recommended. Clearly, the ultimate selection will have implications for facility size and configuration. Instrumentation applied to scientific analyses of martian meteorites have included state-of-the-art scanning electron and transmission electron microscopes (SEM, TEM), ion microprobes and laser-desorption mass spectrometers used for

chemical and isotopic analysis of sub-micron size features. Some members of the scientific community worry that requiring all analytical work be done behind a bio-barrier will restrict access to samples and state-of-art instrumentation, resulting in poorer science return. Currently, there is serious discussion about what analyses must first be conducted behind the bio-barrier and what analyses, if any, can be done outside the barrier on sterilized subsamples.

If evidence of life and/or hazard are found in samples, the facility itself will take on added importance as the ultimate barrier between the samples and the Earth's biosphere. Accordingly, if biological entities are detected, the facility design must be able to accommodate further, more complex testing and analyses. The laboratory must be suitable for determining the source of the life (martian or terrestrial contamination) and the level of hazard posed. In anticipation of such an outcome, the facility must be prepared to consider whether and how portions of the samples may be sent to other laboratories, either in highly secure containers or following biological sterilization.

## CONCLUSION

Returning Martian samples to Earth will be a momentous scientific and technological advance that must be accomplished carefully and responsibly. Planetary protection and biosafety measures are required by international policies and law. Such measures are well justified, both by what is known and unknown about possible extraterrestrial life. Solving the challenges of containment will be critical to mission success not only for Mars missions, but ultimately for sample returns from other bodies of biological interest within the solar system. Doing everything right is important for many reasons—for protecting researchers and the biosphere; for maintaining the integrity of the samples for a wide variety of studies; and for fostering public confidence and support for this exciting human achievement.

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