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# **Mars Sample Handling Protocol Workshop Series**

Interim Report of the Workshop Series  
Workshop 1 Proceedings and Final Report  
Bethesda, Maryland  
March 20-22, 2000

*Edited by:*

*Margaret S. Race  
SETI Institute*

*John D. Rummel  
NASA Headquarters*

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October 2000

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**October 2000**

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## PREFACE

Numerous NASA reports and studies have identified Planetary Protection (PP) as an important part of a Mars Sample Return mission. The mission architecture, hardware, and activities must be designed in ways that prevent both forward- and back-contamination, and ensure maximal return of scientific information. A key element of planetary protection for sample return missions is the development of guidelines for returned sample containment and 'biomarker' analysis.

In 1997, a Mars Sample Quarantine Protocol Workshop [DeVincenzi *et al.* 1999] was convened at NASA Ames Research Center to deal with three specific aspects of the initial handling of a returned Mars sample: 1) biocontainment, to prevent 'uncontrolled release' of sample material into the terrestrial environment; 2) life detection, to examine the sample for evidence of organisms; and 3) biohazard testing, to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere. In 1999, a study by NASA's Mars Sample Handling and Requirements Panel (MSHARP) [Carr, *et al.* 1999] addressed three other specific areas in anticipation of returning samples from Mars: 1) sample collection and transport back to Earth; 2) certification of the samples as non-hazardous; and 3) sample receiving, curation, and distribution.

To further refine the requirements for sample hazard testing and the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened an additional series of workshops beginning in March 2000. The overall objective of these workshops is to develop comprehensive protocols to assess whether the returned materials contain any biological hazards, and to safeguard the purity of the samples from possible terrestrial contamination. This document is the report of the first Workshop in this additional Workshop Series. The information herein will ultimately be integrated into a final document from the entire Workshop Series along with additional information and recommendations (see pages 9 and 13 for further comment).



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## EXECUTIVE SUMMARY

In anticipation of a Mars sample return mission sometime in the next decade, it will be necessary to prepare for handling and testing of martian materials here on the Earth. Previous groups and committees have studied selected aspects of sample return activities, but specific detailed protocols for handling and testing must still be developed. To further refine the requirements for sample hazard testing and to develop the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened a series of workshops beginning in 2000. The overall objective of the Workshop Series is to develop comprehensive draft protocols by which returned martian sample materials could be assessed for biological hazards and to safeguard sample purity from possible terrestrial contaminants.

This document is the report resulting from the first Workshop of the Series, which was held in Bethesda, Maryland on March 20-22, 2000. This report serves to document the proceedings of Workshop 1; it summarizes relevant background information, provides an overview of the deliberations to date, and helps frame issues that will need further attention or resolution in upcoming workshops. Specific recommendations are not part of this report.

Individual Sub-groups were created during Workshop 1 to discuss specific assigned topics. The views and findings expressed by these Sub-groups are preliminary in nature and are not intended to represent a consensus of all participants of Workshop 1. Furthermore, the findings reported herein may not be consistent with the final report and recommendations to be issued at the conclusion of the entire Workshop Series. Although the goal of developing an actual sample-handling protocol is still a long way off, there are areas of consensus emerging, which will be helpful towards that end. To date, the preliminary deliberations and findings of the Sub-groups from Workshop 1 are summarized here (the complete Sub-group reports are included in this document beginning on page 13).<sup>1</sup>

### Sub-group 1: Preliminary Sample Characterization Requirements

Sub-group 1 identified specific data and information that should be collected or recorded about the samples in order to facilitate maximum scientific information. This Sub-group specified that the data should include: information related to the collection site itself, physical characteristics of each specimen, microscopic examination and cross-sections, elemental abundances, mineralogical characterization, non-destructive evaluation of cracks and defects in rock samples, surface reactivity and chemistry, and evaluation of total and organic carbon. In addition, Sub-group 1 highlighted the critical need for further discussions on questions about sterilization of sub-samples<sup>2</sup> prior to their distribution.

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1. During the Workshop, all participants were divided into Sub-groups based on their background and area(s) of expertise and the assigned topics to be discussed. On Day 1, the Sub-groups met for approximately 2 hours. On Day 2, participants were divided into 3 new Sub-groups which met for day-long, in-depth discussions; these same Sub-groups also met on the morning of Day 3 before reporting a summary of their deliberations to the entire Workshop in a final Plenary session.
  2. According to the Space Studies Board (SSB), Task Group on Issues in Sample Return, Mars Sample Return: Issues and Recommendations, National Academy Press, Washington, D.C. (1997), "... if any portion of the sample is removed (from containment) prior to completion of ... analyses, it should first be sterilized." (p. 4). To date, no decisions have been made about sterilization of sub-samples, including the method(s) to be used. At this time, plans are underway to organize a separate Workshop specifically to address questions and issues about sterilization of returned martian sample materials. Any mention of sterilization in this document is based on an acknowledgement that some sub-samples of martian materials may be sterilized and released from containment to perform tests that are part of the overall protocol.

**Combined Sub-groups 2 and 4: Sub-group 2: Representative Sub-samples; Nature of Sample;  
Sub-group 4: Physical/Chemical Analyses; Methods, Sample State, Containment, and Controls**

Although Sub-groups 2 and 4 met separately and were assigned two different discussion topics, they decided to prepare a joint report. Because of their areas of expertise, the members of these two Sub-groups overlapped to a great degree; moreover, the discussions complemented each other because of the focus on the nature and characterization of incoming samples. For the purpose of their combined written summary, they retroactively revised their separate charters to read as one combined charter, as follows:

*"Establish a protocol for documenting, sub-dividing, and characterizing the samples; specifying the nature and sequence of physical, chemical, and mineralogic tests necessary to support the tasks of life detection, biohazard analysis, and preliminary examination for the benefit of the scientific user community."*

The combined Sub-group also proposed a set of operating principles, which they recommend be applied to all activities within the Sample Receiving Facility (SRF). These principles, which represent a concise statement of issues discussed during their sessions (particularly during the discussions by Sub-group 4), include recommendations that all tests be done with the absolute minimum amount of sample necessary; that handling, testing, and characterization activities do the least harm to the returned martian materials; and that geochemical and mineralogic analyses be kept to the minimum necessary to support the protocol.

Sub-groups 2 and 4 constructed a proposed protocol flow chart (see figure 1, page 18) for sample characterization and subdivision, dividing the process into five separate steps that dealt with all three categories of samples (e.g., atmosphere, fines, and rocks). The steps in their process include:

1. Sample Removal and Basic Documentation: extracting and filtering the gas; opening the sample container, removing the sample, and recording basic physical, photographic and curatorial information.
2. Preliminary Characterization: selection of representative sample materials for testing purposes via preliminary visual and gross geological/mineralogical examination, followed by selected non-destructive and non-invasive methods to characterize individual samples; and finally, some fraction of materials selected for testing, while a remaining fraction is stored for future scientific research.
3. Splitting: separating sample types by size fractions or other criteria for use in current protocol testing and/or future scientific testing; sample types distinguished as fines, pebbles, rock cores, and complex pebbles/rocks.
4. Detailed Examination and Analysis (physical chemistry and mineralogy only): analyses to include bulk chemistry, mineralogy, total carbon, preliminary organic carbon analyses, total water assay, and petrography.
5. Release from Containment: samples will either be sterilized or released from containment for controlled distribution, depending upon results from protocol tests.

**Sub-group 3: Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria**

This Sub-group was charged with addressing the end-to-end requirements of an effective sample-testing protocol, using the strawman protocol as a point of departure. Nonetheless, the write-up from Sub-group 3 focused primarily on biohazard assessment, biohazard clearance (i.e., determination of the absence of any biohazard), and the criteria upon which martian samples could be released to the scientific community.

Sub-group 3 reported four particular constraints and working assumptions to be applied to their sample-handling protocol as developed during their deliberations. These were:

1. Any genuine martian life form if found should be kept under continued containment whether it is hazardous or not;
2. Toxicity should be tested, but it is not a criterion for release;
3. Life detection and biohazard testing partially overlap; and
4. Biohazard testing should explicitly emphasize analytic probes that can identify agents that might live, replicate, or otherwise interact with terrestrial carbon-based systems.

The Sub-group specified four levels of questions and methodological approaches that should guide the biohazard testing process, leading to decisions about whether to release materials from containment. These levels included the sequential search for structural indications of life forms, chemical signatures of life, evidence of replication, and monitoring for adverse effects on personnel and the environment at the receiving facility.

Finally, Sub-group 3 highlighted four areas needing further attention:

1. Additional input from other government agencies with experience in biohazard testing;
2. Deliberations on what selection of cell and whole organism types should be used in biohazard assessment;
3. Involvement of statistical experts in assessing the validity of sampling and testing plans;
4. Research and consulting on development of micro-scale model systems for assessing potential impacts on ecosystems.

**Sub-group 5: Candidate Life Detection Tests- Qualifiers, Contraindications, Controls, and Characterization**

Sub-group 5 focused on preliminary identification measurements and tests that should be performed to look for evidence of life or life-related molecules. This Sub-group outlined a series of procedures that will minimally be required to assess for the presence of non-terrestrial life forms in returned martian samples (rocks, soils, and fines). This proposed scheme included initial processing in a nitrogen gas environment at 15°C under strict biocontainment. The Sub-group devised a flow chart (see figure 2, page 24) that suggests sequential processing of various sample types using filtration, fluorescent activated flow cytometry, laser Raman mass spectroscopy, Limulus Amebocyte Lysate (LAL) assays, polymerase chain reaction (PCR) sequencing, micro-scale culturing, broad band fluorescence, and 3-dimensional tomography in a synchrotron. Other analyses that were proposed included tests for chirality and a combination

of capillary electrophoresis, stains, and fluorimetry. Finally, Sub-group 5 suggested that if a survey of samples reveals the absence of carbon or complex organics, the samples can and should be released from the containment facility. If there are indications of biological molecules, more extended testing would, of course, be required.<sup>3</sup>

#### **Sub-group 6: Candidate Biohazard Tests: Qualifiers, Contraindications, Controls, and Characterization**

Sub-group 6 sought to determine the preliminary identification of measurements and tests that should be applied to the sample to analyze for biohazards, without regard to evidence of life or life-related molecules within the samples. Sub-group 6 suggested the need for preliminary testing to gather baseline information on the various sample types, including descriptive and physical characteristics, comparative gas analyses, and X-ray imaging and 3-dimensional image analysis using a synchrotron for carbon analyses. Subsequent to the preliminary data collection, the group proposed a stepwise process to be implemented for biohazard analysis using *in vitro* and *in vivo* testing protocols (see figure 3, page 27).

For *in vitro* testing, the group suggested employing primary and established cell lines derived from plants, animals, insects, humans, bacterial and uni-cellular eucaryotic cell cultures (see Sub-group 6 report, page 25 for further details), and if available, microbial community ecosystem models. Tests for possible biohazards should focus on detecting replicative properties of the hazardous entity, selected phenotypic responses, and host-gene expression responses. For *in vivo* testing, the Sub-group suggested using varied model systems including mouse (e.g., knockout mice with immune defects and Specific Pathogen Free (SPF) out-bred mice), plants (e.g., *Arabidopsis* and others), as well as insect and ecosystem models (details TBD). The group also developed two separate decision trees outlining alternative procedural approaches for the biohazard analysis process (see figures 4 and 5, pages 30 and 31).

Upon completion of the *in vitro*, *in vivo*, and model ecosystem testing, the Sub-group agreed that sample(s) may be selected for release from maximum containment if no biohazard or life form has been detected. The Sub-group suggested, however, that additional experiments and life detection tests be done under level 3 biocontainment subject to case-by-case peer review by an appropriate evaluation panel. Finally, if sub-samples are to be released prior to completion of the protocol testing, the Sub-group stated that the sub-samples should be subjected to extensive gamma irradiation sterilization (dose and time TBD).<sup>4</sup> The group noted that considerable research will have to be done to determine the efficacy of various sterilization methods.<sup>5</sup>

3. To date, no decisions have been made about when and under what conditions sample materials will be eligible for or will actually be released from containment at the Sample Return Facility (SRF). Such decisions will be discussed in later Workshops and will invariably involve considerations of sample sterilization and interpretation of protocol test results. Ultimately, it is likely that decisions about what is done with sample materials will be made after review by an appropriate international scientific oversight committee at the SRF in consultation with NASA's Planetary Protection Officer and other responsible officials.
4. To date, no decisions have been made about sterilization of sub-samples, including the method(s) to be used.
5. At this time, plans are underway to organize a separate Workshop specifically to address questions and issues about sterilization of returned martian sample materials.



## Notes

*This document is the final report of Workshop 1, but only an interim report of the Workshop Series. This report is intended to provide a summary of Workshop 1 to serve as background information for participants of future workshops in the Series and any other interested parties. It will also serve as a starting point for deliberations during Workshop 2 (see page 9 for further comments on this topic). If any portion of this report is to be cited or referenced it must be with the understanding that this document is neither authoritative nor indicative of any final decisions or plans for future Mars missions.*

This Executive Summary was drafted from summaries written by each Sub-group following Workshop 1. The complete summaries, which appear in the main body of this report, have undergone minimal editing. No attempt has been made to reconcile differences between the Sub-groups, nor to determine at this time whether particular suggestions would be feasible or recommended for a Mars sample return mission. Throughout this report, the reader is referred to 'notes' which serve to qualify or clarify the temporary nature of particular statements; these notes appear in Appendix G. The collective thoughts and suggestions of all the Sub-groups will be subject to further discussion at future workshops. The information herein will eventually be integrated with additional findings and recommendations from the entire Workshop Series. Upon completion of the Workshop Series, a final report for the Series will be published.



## INTRODUCTION

For upcoming Mars sample return missions, NASA is committed to following the recommendations developed by the Space Studies Board (SSB) of the National Research Council (NRC) in its report on sample handling and testing [SSB 1997]. In particular, the NRC recommended that: a) "samples returned from Mars by spacecraft should be contained and treated as potentially hazardous until proven otherwise, and b) "rigorous physical, chemical, and biological analyses [should] confirm that there is no indication of the presence of any exogenous biological entity." As a step towards specifying the requirements for sample hazard testing and the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened a series of workshops in 2000 – 2001. The stated objective for this Workshop Series is:

*"For returned Mars samples, develop a recommended list of comprehensive tests, and their sequential order, that will be performed to fulfill the NRC recommendations that 'rigorous analyses determine that the materials do not contain any biological hazards.'"*

Overall, the Mars Sample Handling Protocol Workshop Series has been designed to touch on a variety of questions such as: "What types/categories of tests (e.g., biohazard, life detection) should be performed upon the samples? What criteria must be satisfied to demonstrate that the samples do not present a biohazard? What constitutes a representative sample to be tested? What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what physical/chemical analyses are required to complement biochemical or biological screening of sample material? Which analyses must be done within containment and which can be accomplished using sterilized material outside of containment? What facility capabilities are required to complete the protocol? What is the minimum amount of time required to complete a hazard-determination protocol? By what process should the protocol be modified to accommodate new technologies that may be brought to practice in the coming years (i.e., from the time that a sample receiving facility would be operational through the subsequent return of the first martian samples?)

The first Workshop in the Series was held in Bethesda, Maryland on March 20-22, 2000 (see Appendix A for the Agenda of Workshop 1). Because the process of developing the protocols necessarily requires input from a wide range of scientific areas, individuals from a variety of institutions and areas of disciplinary expertise were invited to participate (see the Participant Lists in Appendices B1 and B2).

To keep the Workshop focused, a set of basic assumptions were given to guide and constrain deliberations:

1. Regardless of which mission architecture is eventually selected, samples will be returned from martian sites which were selected based on findings and data from the Mars Surveyor program missions.
2. Samples will be returned sometime in the next decade.
3. Samples will not be sterilized prior to return to Earth.

4. When the sample return canister (SRC) is returned to Earth, it will be opened only in a sample receiving facility (SRF) where samples will undergo rigorous testing under containment and quarantine prior to any controlled distribution ('release') for scientific study.
5. The amount of sample to be returned in a SRC is anticipated to be 500-1000g.
6. The sample will likely be a mixture of types including rock cores, pebbles, soil, and atmospheric gases.
7. The amount of sample used to determine if biohazards are present must be the minimum amount necessary.
8. Samples must be handled and processed in such a way as to prevent terrestrial (chemical or biological) contamination.
9. Strict containment of un-sterilized samples will be maintained until quarantine testing for biohazards and life detection is accomplished. Sub-samples of selected materials may be allowed outside containment only if they are sterilized first.
10. The SRF will have the capability to accomplish effective sterilization of sub-samples as needed.
11. The SRF will be operational two years before samples are returned to Earth.
12. The primary objective of the SRF and protocols is to determine whether or not the returned samples constitute a threat to the Earth's biosphere and populations (not science study *per se*) and to contain them until this determination is made.

In order to give all participants a common basis in the technical areas necessary to achieve the objectives of the Workshop, the first part of the Workshop was devoted to tutorial presentations. These presentations covered Mars mission architectures and plans, historical experiences with extraterrestrial sample handling, and relevant reports and recommendations related to planetary protection. Additionally, summaries of key sample return/planetary protection reports were distributed as pre-workshop 1 reading. The summaries of key PP reports are in Appendix C and the tutorial viewgraphs are presented in Appendix D.

For the second part of the Workshop, participants were divided into sub-groups to address six separate assigned topics related to sample handling and testing. The sub-groups were organized to discuss the major issues in each assigned area and to develop recommendations as appropriate. The discussion topics that were assigned to each sub-group are listed below. Topics 1-3 were allotted ~2 hours each for discussion on the afternoon of the first day, while topics 4-6 were covered in greater depth in day-long sub-group sessions on Day 2:

- Topic 1: Preliminary Sample Characterization Requirements
- Topic 2: Representative Sub-samples; Nature of Sample
- Topic 3: Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria
- Topic 4: Physical/Chemical Analyses-Methods, Sample State, Containment, and Controls
- Topic 5: Candidate Life Detection Tests - Qualifiers, Contraindications, Controls, Characterization
- Topic 6: Candidate Biohazard Tests - Qualifiers, Contraindications, Controls, Characterization

On the final day of the Workshop, all participants contributed to an open discussion in plenary session focused on three additional topics: Criteria for Release; Context of Collection; and Single/Multiple containment facilities. These open discussions were helpful in reviewing the various topics and exploring issues that will be discussed further in upcoming workshops. No attempts were made to summarize these discussions at this time.

*This document is the final report of Workshop 1, but only an interim report of the Workshop Series. This report is intended to provide a summary of Workshop 1 and to serve as background information for participants of future workshops in the Series and any other interested parties. It will also serve as a starting point for deliberations during Workshop 2. This report is a record of the complete Workshop 1 process; it contains summaries of key PP reports, the background tutorials presented at Workshop 1 (in the form of the viewgraphs used by the speakers), and summary reports from the six Sub-groups (see Note 1, Appendix G), as well as the agenda and list of participants. Ultimately, the information contained in this report will be integrated with information and recommendations that emerge from the remaining workshops in the Series. A Final Report for the overall Workshop Series will be published at the conclusion of the Series following review by a science advisory group.*

It is hoped that the final report will: 1) assist NASA's Planetary Protection Officer and senior administrators in preparing for Mars sample return facilities, technology, and operations; 2) serve as a briefing document for advisory groups, regulatory agencies, and other entities who will ultimately establish and review sample return handling policies, requirements, and implementation, and 3) provide recommendations in a form suitable as input for possible future announcements of opportunity soliciting proposals for Mars sample handling.



## BACKGROUND TUTORIALS OVERVIEW

The Background Tutorials that were presented at Workshop 1, were designed to address important issues and technical information associated with a Mars sample return mission and subsequent sample handling and testing. The tutorials were presented as the first part of the Workshop in order to give all participants a common basis in the technical areas necessary to achieve the objectives of the Workshop. The viewgraphs of each tutorial can be found in Appendix D (beginning on page 87); the titles of the presentations and presenters' names and affiliations are listed here:

Overview of Mars Sample Hazard Analysis  
*John D. Rummel (NASA Headquarters)*

Planetary Protection Overview  
*John D. Rummel (NASA Headquarters)*

French Participation in Mars Sample Return and Mars Exploration  
*Jean-Louis Counil, (Centre National de la Recherche Scientifique)*

Summary of 1992 and 1997 NRC (Space Studies Board) Task Group Reports  
*Kenneth Nealson (NASA Jet Propulsion Laboratory)*

Mars Sample Return Mission Design  
*Robert Gershman (NASA Jet Propulsion Laboratory)*

Options in Extraterrestrial Sample Handling and Study  
*Dimitri A. Papanastassiou (NASA Jet Propulsion Laboratory)*

Mars Sample Handling and Requirements Panel (MSHARP) Report Summary  
*Donald L. DeVincenzi (NASA Ames Research Center)*

Current State of Controversy about Traces of Ancient Martian Life in Meteorite ALH84001  
*Allan H. Treiman (Lunar and Planetary Institute)*

Lunar Sample Quarantine and Sample Curation  
*Judith H. Allton (Lockheed Martin/NASA Johnson Space Center)*

Summary of 1997 Mars Sample Quarantine Protocol Workshop Report  
*Margaret S. Race (SETI Institute)*

Draft Protocol (A Working Guideline for the Deliberations at Workshop 1)  
*John D. Rummel (NASA Headquarters)*





## SUB-GROUP CHARTERS, MEMBERS, AND REPORTS

During the course of Workshop 1, the participants were divided into sub-groups to discuss particular issues or problems associated with sample handling and testing. Guided by a chairperson and co-chairperson who facilitated the sub-group's deliberations, each sub-group discussed the major issues in their assigned topical area, developed recommendations as appropriate, and reported back to the entire Workshop in subsequent plenary sessions.

On the afternoon of the first day, participants were divided into three sub-groups, each of which focused on one of three key questions relevant to the overall protocol framework. Topics 1-3 were allocated only a brief time for discussion (approximately 2 hours each), before summary reporting in plenary session. The reports of these initial sub-groups were necessarily cursory. The three Day 1 topics were:

1. Preliminary Sample Characterization Requirements
2. Representative Sub-samples; Nature of Sample
3. Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria

During the second day, participants were again assigned to one of three sub-groups, each of which focused on one of three topics. Topics 4-6 were covered in greater depth during the Day 2 break-out session, which lasted a full day. The assigned Day 2 sub-group topics were:

4. Physical/Chemical Analyses - Methods, Sample State, Containment, and Controls.
5. Candidate Life Detection Tests - Qualifiers, Contraindications, Controls, Characterization
6. Candidate Biohazard Tests - Qualifiers, Contraindications, Controls, Characterization

The sections that follow present information on the specific charters assigned to each sub-group, the names of sub-group members, and a summary report of findings for each sub-group. The summary reports reflect the deliberations of the members of each sub-group (see Note 2, Appendix G). The findings are preliminary and there may be inconsistencies among the sub-groups. The views expressed, and any conclusions and recommendations reached by the sub-group reports, *do not* represent a consensus of all Workshop participants, and will not necessarily be consistent with the final report nor with recommendations that will be issued at the conclusion of the Workshop Series (see page 9 for further comments on this topic). This first workshop was productive in setting the stage for the Workshop Series and framing questions that will be addressed in greater detail in subsequent workshops.

Finally, in addition to the six sub-groups topics listed above, three additional topics were discussed by all participants in the plenary session on the third day: criteria for release; context of collection; and single versus multiple containment facilities. These open discussions were helpful in reviewing the various topics and exploring issues that will be discussed further in upcoming workshops. No attempt has been made to summarize these discussions at this time.

### Sub-Group 1: Preliminary Sample Characterization Requirements

Sub-group 1 was given the task of "specifying the information about the samples required to enable effective life-detection and/or biohazard testing. The focus will be on sample characteristics that could be determinative in understanding the results of both *in vitro* and *in vivo* testing that may be required. Example information that may be available or obtainable includes: site of collection on Mars; preservation conditions *en route* to Earth and the sample-containment lab; elemental composition; mineralogical characteristics; mass; volume; etc."

The Members of Sub-group 1 were:

Fishbein, William (Chairperson)  
Maurel, Marie-Christine (Co-Chairperson)  
Cronin, John  
Flandrois, Jean-Pierre  
Friedmann, E. Imre  
Gerba, Charles  
Granges, Jacques  
Johnson, Dale  
Khan, Ali  
Marty, Bernard  
Murphy, William  
Mustin, Christian  
Nealson, Ken  
Pepper, Ian  
Relman, David  
Sogin, Mitchell  
Walker, Robert

Following its discussions, Sub-group 1 indicated that preliminary information essential to sample collection should include the following: the exact geographic location and date/time notation (e.g., *in situ* sample orientation (which way is north), depth of sampled core or fines, etc.), proximity to the lander and risk of pollution, and radiation level and temperature at time of collection and throughout its voyage back to Earth. A small sample of martian atmosphere could serve as a control for assessing possible changes in solid samples arising during storage or transit. The sub-group further suggested that preliminary basic testing of each specimen might include:

1. Radioactivity, mass, volume, density, gross and light microscopic exam with color photos;
2. If feasible, virtual cross-sections of large specimens (rocks or cores) to identify regions for additional study that are most likely to harbor life forms (e.g., prior water, secondary mineralization);
3. Major element abundances (those  $\leq 0.5\%$  total) by X-ray fluorescence;
4. Mineralogical characterization, with preservation of secondary minerals;
5. Evaluation of cracks and other defects in rock samples non-destructively and without sterilizing (X-ray tomography);
6. Surface reactivity and chemistry (organics,  $M^{++}$  oxidation states and potentials); and,
7. Evaluation of total carbon and organic carbon (by stepwise combustion and mass spectroscopy (MS) to  $10^{-14}$ ).

Sub-group 1 noted that one of the main goals of a Mars Sample Return mission in the next decade is the evaluation of returned samples for evidence of possible martian life forms, current and/or extinct. If it is recommended that samples be sterilized prior to any distribution for analysis outside of the containment facility, Sub-group 1 strongly suggested that the sterilization should only be applied to aliquots, never to the whole specimen. A paradox arises in that the finding of life forms which are capable of replication, the most exciting of biologic possibilities, would delay or even prevent the distribution of samples (see Note 3, Appendix G), whereas the absence of such forms would allow distribution to interested scientists (see Note 4, Appendix G). Sterilization of aliquots can be used for distribution, but there is a need for further study to determine the minimal effective sterilization doses for various types of samples (see Note 5, Appendix G). Sub-group 1 indicated concern that sterilization would require doses high enough to decompose macromolecules and other signatures of past or present life forms. Additional research and discussion is needed prior to making recommendations about sterilization methodology and implementation.

#### **Combined Sub-groups 2 and 4: Revised Task**

Although they met separately during the Workshop, Sub-groups 2 and 4 combined their findings following the Workshop and submitted a single report. To reflect this integration, they also revised their charter to be a combination of the two separate charters; the combined charter read as follows: "Establish a protocol for documenting, subdividing, and characterizing the samples; specifying the nature and sequence of physical, chemical, and mineralogic tests necessary to support the tasks of life detection, biohazard analysis and preliminary examination for the benefit of the scientific user community." The original separate assigned charters are described below.

#### **Sub-Group 2: Representative Sub-samples; Nature of Sample**

Initially, Sub-group 2 was given the task of "specifying the preliminary characterization data that would be required to enable partitioning of the entire body of returned samples into representative sub-sample aliquots for testing. Additionally, they were asked to recommend a process whereby returned samples could be sub-sampled effectively. This Sub-group was also asked to specify which information about the samples should be obtained within containment, either to support time-critical sample characterization and distribution for later scientific analysis, or to understand the requirements for curation of the samples.

The Members of Sub-group 2 were:

MacPherson, Glenn (Chairperson)  
Bibring, Jean-Pierre (Co-Chairperson)  
Allen, Carl  
Allton, Judith  
Bogard, Donald  
Bradley, John  
Des Marais, David  
Holland, Heinrich  
Papanastassiou, Dimitri  
Pavé, Alain  
Prieur, Daniel  
Treiman, Alan

Vasil, Indra  
Wainwright, Norman

#### **Sub-Group 4: Physical/Chemical Analyses-Methods, Sample State, Containment, and Controls**

Initially, Sub-group 4 was given the task of "addressing desired methods to conduct physical and chemical analyses of the sample and sub-samples to meet the requirements of the sample-analysis protocol, curation, and storage. Methods will be assessed for their ability to obtain the required information while minimizing destruction of the samples tested, and as to their ability to be performed inside of the containment facility or on sterilized samples (sterilization methods TBD) outside of containment."

The Members of Sub-group 4 were:

Bogard, Donald (Chairperson)  
Marty, Bernard (Co-Chairperson)  
Allen, Carl  
Allton, Judith  
Bibring, Jean-Pierre  
Bradley, John  
Cronin, John  
Holland, Heinrich  
Johnson, Dale  
MacPherson, Glenn  
Mustin, Christian  
Papanastassiou, Dimitri  
Treiman, Alan  
Walker, Robert

#### **Combined Sub-group 2/4: Findings**

In devising the sequence and nature of tests and the overall sample flow, and in conducting preliminary scientific examination of the samples, combined Sub-group 2/4 recommended that the following 'operating principles' *must* underlie all activities within the receiving facility:

1. All tests and preliminary characterization activities must use the absolute minimum amount of sample which is necessary to successfully carry out the test (see Note 6, Appendix G).
2. All handling, tests and preliminary characterization activities should aim to do the least harm to any and all samples (i.e., non-destructive, non-invasive tests are preferable).
3. If the samples are returned cold from Mars, processing and storage must be done cold (temperature TBD). Also, a non-harmful environment (dry?) filled with a non-contaminating gas (TBD) is required.
4. Geochemical and mineralogic analyses should be kept to the minimum required to fully support biohazard assessment, life detection, and basic characterization for the purpose of future sample allocations.

The details of many procedures listed in this report are yet to be decided, but are very important. They will require far more careful deliberation than was possible during the short duration of the Workshop. These are noted as 'to be determined' (TBD).

The following proposed protocol for sample handling and testing accompanies the flow chart shown on the next page (see figure 1). The protocol defines three categories of samples: atmosphere, fines (i.e., 'soils'), and rock; rock is further subdivided into pebbles and rock cores. All sample types are treated somewhat differently in the proposed protocol outlined below:

1. **Sample Removal and Basic Documentation:** Upon receipt of the sample container, the ambient martian atmosphere is extracted (method TBD) and filtered for possible suspended matter. The filtered particulate samples are set aside for testing. The gas sample is subdivided (method TBD) and retained for later allocation.

Once the sample container is opened, the individual samples are removed, identified, and correlated with lander/rover sample numbers, weighed, photographed, assigned identification numbers, and the data recorded. Dust from the inner surfaces of the sample container, from the surfaces of the individual sample tubes, and from the surfaces of the samples themselves is collected and set aside for testing.

2. **Preliminary Characterization:** It is desirable at this stage to select samples that are representative, and also to identify for testing samples that are most likely to contain signs of ancient or extant life (e.g. sedimentary rocks, or those with elevated water contents).

Individual samples of fines, pebbles, and cores are examined, described visually, and photographed in detail. Individual rocks are characterized as being igneous, sedimentary, or breccias. 'Complex rocks,' such as those containing unusual clasts or veins, are specifically identified. The extent and physical nature of any weathering or alteration is noted. Some attempt should be made to group similar rock types, as one way of identifying representative sub-samples for the required biohazard/life assessment tests.

For each sample of fines it is desirable to estimate the mean grain size and if possible, the nature of abundant individual grains. Individual larger (e.g., greater than several millimeters – exact size TBD) rock fragments should be separated from the soils and be treated as individual samples.

To enable such characterization, individual samples may be analyzed using such non-destructive and non-invasive methods as high-powered visual optics, IR and UV spectroscopy, and qualitative X-ray fluorescence analysis. IR in particular may be useful for quickly identifying samples with elevated water contents. Where feasible, these tests should be performed through a window of the processing cabinet, or at least be made with the maximum amount of instrumentation located outside the cabinet.

Based on these preliminary examinations and tests, some fraction of the samples (fraction TBD) is selected for testing. The remaining fraction is stored for posterity ('The Bank') and not touched or characterized at this time.

3. **Splitting:** Samples of fines are separated into two size fractions (less than 1mm and greater than 1mm), by sieving. The different fines samples and atmospheric dust filtrates are split into fractions (TBD) for detailed physical chemical characterization, biohazard determination, and life detection. The remainder is stored. Simple pebbles and rock cores are similarly split (splitting method TBD).

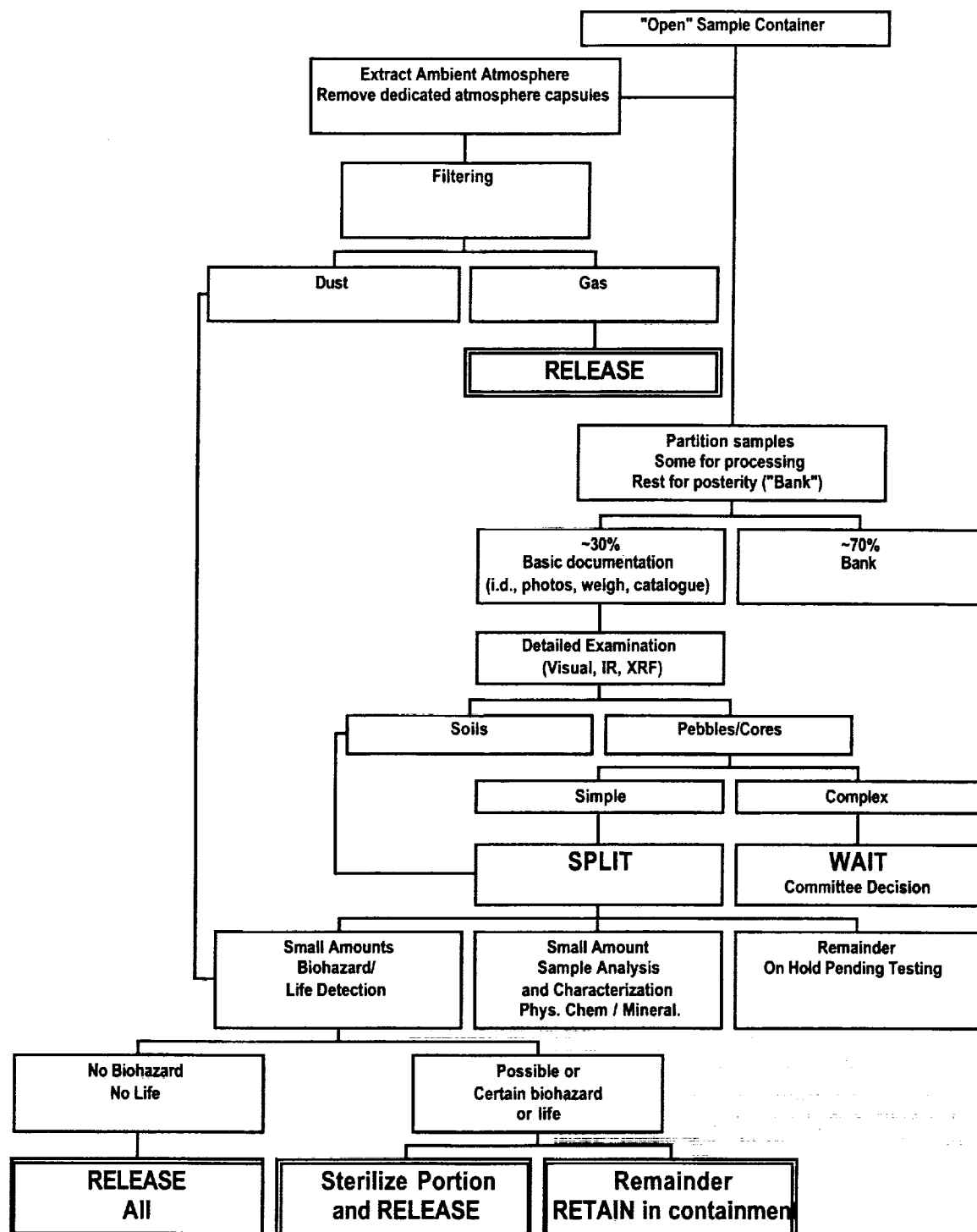


Figure 1: Proposed Sample Characterization and Subdivision Proposal  
(Sample Handling and Testing Protocol)

Complex pebbles and rock cores are not immediately split. Complex rocks may contain small sub-lithologies of materials that are of special interest both for long-term research purposes and for immediate biohazard/life detection purposes. This material is very precious. Splitting and sampling of such materials must be done only after considerable thought and consultation between a multinational oversight team of biologists and geologists. Based on these careful deliberations the science oversight team will make final decisions about how to split and sub-sample complex rocks most effectively. Once a decision is made regarding special lithology sampling and what constitutes a representative sub-sample of complex rocks, splitting is done accordingly and as above.

4. Detailed Examination and Analysis (Physical chemistry and mineralogy only): Certain tests were seen as required; where these tests should be done was a matter of controversy at the Workshop, and was not resolved (see Note 7, Appendix G). This must be addressed further. The following tests are required:
  - Bulk chemistry (wavelength dispersive X-ray fluorescence); determine all major elements (>1% abundance) and some minor elements (<1%: Mn, Cr, Ti, Ni, Na, K, P; others?).
  - Mineralogy (X-ray diffraction); determine major minerals within samples.
  - Total carbon by stepped combustion (this will simultaneously give fraction inorganic carbon and fraction organic carbon from combustion temperature ranges).
  - Preliminary analysis of organic carbon by stepped pyrolysis and gas chromatography, high-resolution mass spectrometry. Furthermore, specific organic analyses (TBD) in consultation with biologists and the science advisory committee at the receiving facility, taking into account the results of life detection and biohazard analyses, the amount and nature of organic carbon found, method sensitivity, and the resulting sample size requirements. Assay of total water may be built into this analysis as well. Because of the likely degradation of organic compounds by sample sterilization, the organic measurements should be performed using highly capable instrumentation located within the containment facility.
  - Total water assay (instrumentation TBD; possibly done as part of organic carbon analysis, above).
  - Petrography; thin sections to be made of only a subset of samples, perhaps as small as 10%. (X-ray fluorescence and X-ray diffraction are the clear methods of choice for chemistry and bulk mineralogy: The techniques are mature and well understood, rapid, sensitive, precise, and require instrumentation that is relatively small and simple to operate.)
5. Release from Containment/Dispensation: Depending on results of biohazard and life detection tests, remaining portions of samples will either be released for allocation outright, or sterilized and then released for allocation (sterilization method TBD, but must be done in such a way as not to destroy the scientific value of the samples; any heating is especially undesirable) (see Notes 4 and 5, Appendix G).

### **Sub-Group 3: Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria**

This Sub-group was assigned the task of "addressing the end-to-end requirements of an effective sample-testing protocol. The strawman protocol may be used as a departure, or the Sub-group may define its own strawman protocol. Attention will be given to the sequence of testing, the timing and availability of complementary test results to support other testing anticipated in the process, and the nature of the criteria that shall be met to enable sample release (e.g., controlled distribution), for scientific analysis. To the extent possible, an end-to-end protocol should be blocked out for further discussion."

The Members of Sub-group 3 were:

Jahrling, Peter (Chairperson)  
Sourdive, David (Co-Chairperson)  
Candresse, Thierry  
Chyba, Christopher  
Crissman, Harry  
Eisen, Jonathan  
Fultz, Patricia  
Gabriel, Dean  
Hawley, Robert  
Kovacs, Gregory  
Leonard, Debra  
Moutou, François  
Persing, David  
Richmond, Jonathan  
Tennant, Raymond  
Viso, Michel  
Wall, Diana

In the initial assignment, Sub-group 3 was given the task of “addressing the ‘end-to-end’ requirements of an effective sample testing protocol.” However, following its discussions, the Sub-group’s write-up focused primarily on biohazard assessment, ‘biohazard clearance’ (i.e., determination of the absence of any biohazard), and the criteria upon which martian samples could be released (i.e., distributed) to the scientific community. It also attempted to clarify what questions should be answered by the sequence of tests performed for biohazard clearance.

Sub-group 3 adopted an approach consistent with the Space Studies Board recommendations [SSB 1997] for returned martian materials. For discussion purposes, they specified the following: Samples returned from Mars have to be clean, contained, and sterilized: *clean*, as in not contaminated with terrestrial organisms; *contained* to prevent contamination of the Earth’s biosphere; and *sterilized* if any portion of the sample is removed from containment for further analysis prior to completion of the rigorous analyses (see Notes 4 and 5, Appendix G). In addition, the Sub-group highlighted the following constraints and assumptions:

1. Any genuine martian life will be contained.  
The Sub-group acknowledges that should the samples contain any genuine active martian form of life, *be it hazardous or not*, then the samples should be kept under appropriate level of containment, or be thoroughly sterilized before release.<sup>6</sup>
2. Toxicity should be tested, but it is not a criterion for release.  
In addition to testing samples for evidence of replicating life forms, Sub-group 3 noted the importance of screening for agents in returned samples that might be toxic to Earth life-forms, such as hot radioactive particles and chemical toxins. Testing of samples should therefore include both biohazard and toxicity assessment:
  - Screening for radioactivity;
  - Potential chemical hazards;
  - Toxicity for bacterial and eukaryotic cells;
  - Search for replication in enriched media (liquid/solid);
  - Effect/growth on various cell cultures;

6. Consistent with recommendations made in the 1997 SSB report on Mars Sample Return.



- Effect/growth on whole organisms (i.e., murine/specified rodent; plant); and,
- Effect on the biosphere.

However, Sub-group 3 unequivocally asserted that absence of possible toxicity should not be *per se* a criterion for release. All facilities housing or manipulating samples should be informed of any radioactivity or toxicity in the sample and appropriate personnel protective measures should be taken accordingly. Only evidence of real biohazards or genuine active martian life forms should be regarded as relevant criteria for decisions about releasing or not releasing any un-sterilized samples.

3. Search for martian life forms partially overlaps biohazard testing.  
Sub-group 3 recognized that biohazard and life detection testing can partially overlap. Close cooperation with the life-detection team is essential to reduce unnecessary duplication of effort or redundant destruction of sample, and to reduce the time required to make the decision regarding release from containment.

However, while the life-detection team will examine samples for evidence of possible biological entities or activity under past or present martian conditions, the biohazard testing will focus solely on any dangers posed by a possible release of an alien life form on Earth, under terrestrial conditions (i.e., not martian conditions).

4. Biohazard testing relies on the 'carbon assumption.'  
Consistent with the logic used by the SSB Task Group on Sample Return [SSB 1997], Sub-group 3 suggested that biohazard testing should focus on self-replicating entities capable of propagating on Earth and possibly interacting with people, animals, plants, or microbes. The Sub-group explicitly acknowledged that it could not envision any kind of biohazard that does not explicitly or implicitly rely on the 'carbon assumption,' that is, being structurally based on carbon chemistry as we know it on Earth. Primary interest in biohazard testing will thus be to identify agents that might live, replicate, or otherwise interact with terrestrial carbon-based systems.

In its deliberations, Sub-group 3 also identified the following concerns or issues to be resolved:

1. In developing and reviewing the proposed testing protocol, input is needed from a broad range of agencies with responsibilities and experience in biohazard testing (e.g., U.S. Department of Agriculture, U.S. Environmental Protection Agency, U.S. Department of the Interior, relevant international agencies, etc.)
2. In the process of designing the testing protocols, additional discussions are needed on what selection of cell and whole organism types should be used in biohazard assessments.
3. Because of the anticipated difficulty of determining what will constitute representative samples, controls and replicates, there is a critical need to involve statistical experts in assessing the validity of proposed sampling and testing plans;
4. Currently, it appears that no micro-scale model systems are available for assessing potential impacts on ecological and biosphere systems. Sub-group 3 identified this as an important area for consultation and directed research.

Sub-group 3 developed a draft chart outlining a sequence of questions that would be important to answer before determining whether to release materials from biocontainment. Table 1 indicates four levels of questions relevant to biohazard assessment, and possible testing strategies for answering the questions.

Item	Question	Strategy
1	Is there anything that looks like a life-form?	Beam synchrotron or other non-destructive high-resolution analytic probe, particularly one that would allow testing non-sterilized (yet still contained!) samples outside main facility.
2	Is there a chemical signature of life?	Mass spec. or other test systems (to be used in containment) that would identify asymmetry, special bonding, etc.
3	Is there any evidence of self replication or replication in terrestrial living organism?	Attempts to grow in culture or in cell culture, defined living organisms.
4	Is there any adverse effect on workers or the surrounding environment?	Medical surveillance; evaluation of living systems in proximity of the receiving facility

Table 1: Sequence of Questions and Possible Strategies  
for Decisions about Release of Sample Material from Containment

#### Sub-Group 5: Candidate Life Detection Tests-Qualifiers, Contraindications, Controls, and Characterization

Sub-group 5 was assigned the task of "preliminary identification of measurements and tests that should be applied to the samples to look for evidence of life or life-related molecules. Methods and instrumentation to be used should be identified to the extent possible. The relationships of the information to be gained from complementary life-detection tests should be specified to the degree possible. This Sub-group will recommend methods and concepts to be discussed at a later workshop in support of protocol development."

The Members of Sub-group 5 were:

Sogin, Mitchell (Chairperson)  
 Prieur, Daniel (Co-Chairperson)  
 Crissman, Harry  
 Des Marais, David  
 Flandrois, Jean-Pierre  
 Friedmann, E. Imre  
 Maurel, Marie  
 Nealson, Ken  
 Pavé, Alain  
 Pepper, Ian  
 Persing, David  
 Wainwright, Norman

Sub-group 5 focused its discussions on detection of life or life-related molecules in samples returned from the planet Mars. The challenge facing scientists and technicians in the Mars sample return quarantine facility will be to efficiently determine whether there is evidence of

life, viable or dead, in a variety of samples without compromising their pristine nature or consuming significant amounts of samples to perform the analyses.

This Sub-group identified three possible outcomes of the life detection procedures. The first is demonstration that no terrestrial-like life exists as evidenced by the complete absence of carbon or complex carbon in the returned sample. This result would lead to considering the release of tested samples from the quarantine facility. The second would be clear and overwhelming evidence of living organisms as demonstrated by self-replicating entities capable of evolving (see Note 8, Appendix G). Discovering life forms of non-terrestrial origins would be profound and would likely dictate containment for an indefinite period of time. Furthermore, such a result would likely require the samples to be reserved for biological experimentation. The third and most likely scenario lies between extremes where complex carbon containing compounds are present in the sample, but without clear evidence of replicative properties.

The current mission plan will return three kinds of samples including gas, small particles or fines (particles less than 2000 microns in size) and pebbles or larger fragments (including cores). Each kind of sample will require different processing procedures that must obtain as much information as possible with minimal destruction/consumption of the sample. A series of tests will be required to evaluate the likelihood that a particular sample contains or once contained life forms. It will be also be necessary to design experiments to determine whether or not the samples are potentially hazardous to animals, fungi, plants, and microorganisms making up our biosphere.

Based on its discussions, Sub-group 5 outlined a series of procedures that will be minimally required to assess the presence of non-terrestrial life forms in returned martian samples (see Note 9, Appendix G). The time required to complete analysis prior to relaxing sample quarantine requirements is difficult to predict, however every effort should be made to expedite the process without compromising the possible outcomes. The worst-case scenario would be to overlook the occurrence of non-terrestrial life forms. The penultimate worst-case scenario would be to mistakenly identify terrestrial contamination as being evidence of non-terrestrial life (false positives). Experimental rigor is of paramount importance in the initial analyses of the samples.

To detect life forms in samples returned from Mars, the Sub-group recommended employing techniques that are able to detect low concentrations of organisms or molecular species of potential biological origin. They must be able to efficiently scan large surfaces or large numbers of particles over which a small number of biological entities might be distributed. To maintain the samples in pristine condition, the initial processing should occur in a nitrogen gas environment at 15°C. Procedures and instrumentation employed must be compatible with the highest-level biosafety facility (i.e., BSL-4), although procedures requiring facilities outside of the containment facility will be possible if the sample can be maintained in a sealed container. When possible, initial characterizations should be non-destructive so that the sample can be retained in a near pristine state for physical characterizations. This requirement can be met for fines greater than 2000 microns in size, and uncomplicated pebbles and rocks (i.e., pebbles or rocks without cracks or pores). For gases, complicated samples, and fines less than 2000 microns in size, minimal amounts of representative samples will be subjected to destructive tests according to the flow chart outlined in figure 2.

Nitrogen Gas Environment

15 C

1 mg/sample

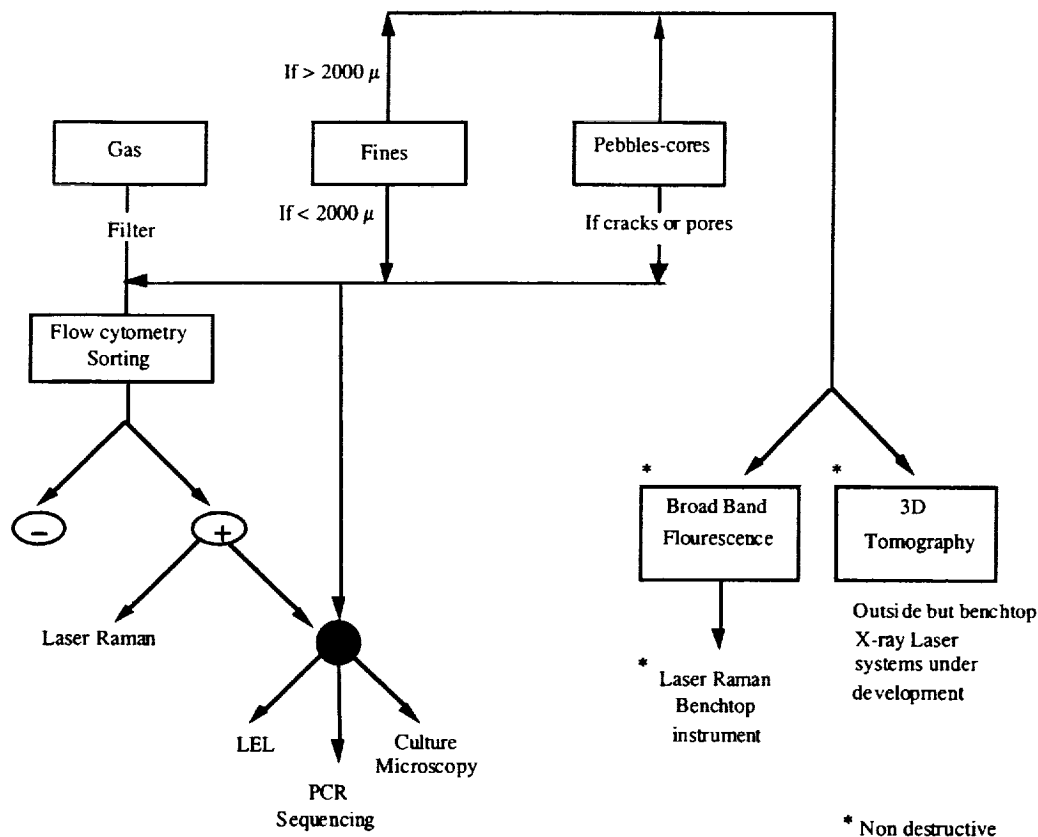


Figure 2: Proposed Sample Handling and Life Detection Testing

In the flow chart, particles collected from filtered gas samples, small particles (i.e., less than 2000 microns), and microscopic by-products from any core samples that might be taken from complex pebbles or rocks, will be sorted by a fluorescent activated flow cytometer. Biological systems usually have fluorescent molecules that can serve as the basis for sorting particles. Contemporary machines are capable of physically sorting particles at rates of hundreds- to tens-of-thousands per second. The sorted and unsorted sample material will be recovered for more detailed analyses. Portions of all samples will be subjected to combustion analysis and mass spectroscopy (MS) to detect the presence or absence of carbon and to identify complex reduced organics, sulfur, and nitrogen. The sensitivities of bench top MS are will within the range required to detect small concentrations of complex molecules.

Positive samples from the cell sorting, as well as cores and shavings from complex pebbles and rocks, will be further studied using light microscopy, LAL assays, and PCR/sequencing. These studies will be complemented by limited attempts to culture organisms at the micro-scale level. Micro-scale culture enrichment techniques are just beginning to emerge and funds will be required for further development of this technology. Other tests that will be applied include

assays for amino acids and proteins using tests for chirality and a combination of capillary electrophoresis systems, stains, and fluorimetry. Nucleic acids including DNA, RNA, and PNA will be assayed using fluorescence microscopy, fluorimeters, PCR techniques, and electrophoresis. Membranes and cell walls (e.g., fatty acids) can most efficiently be detected using MS.

Fines greater than 2000 microns and uncomplicated pebbles or rocks (lacking pores and cracks) will be studied using broad band fluorescence and 3-dimensional tomography in the synchrotron (using sealed samples). Broad band fluorescence allows surveys of large surfaces in a short period of time, while 3-dimensional tomography at lower energies allows detection of mineralogy that is indicative of biology with minimal impact on the samples. If broad band fluorescence provides positive signals, non-invasive Laser Raman spectroscopy can identify classes of UV-absorbing molecular species (e.g., double-bonded carbon). These techniques will permit inspection of samples with minimal perturbation.

Except for the synchrotron, all of these techniques can be used inside of a BSL-4 facility but only culturing techniques can provide absolute evidence of a biological entity that must be contained for indefinite periods of time. If a survey of samples reveals the absence of carbon or complex organics, the Sub-group recommended that the samples can and should be released rapidly from the containment facility (see Note 4, Appendix G). If there are indications of biological molecules including proteins, nucleic acids and chirality, life detection will require more extended testing and there will be a requirement to evaluate the potential biohazard of the samples both to multicellular species and to the environment via bioassay tests TBD. This latter requirement may pose the most difficult challenge for studying the potential impact of samples returned from Mars on our biosphere.

#### **Sub-Group 6: Candidate Biohazard Tests-Qualifiers, Contraindications, Controls, and Characterization**

Sub-group 6 was assigned the task of "preliminary identification of measurements and tests that should be applied to the samples to test for biohazards that may be present in the samples, without regard to evidence of life or life-related molecules within the samples. Methods, test systems, and instrumentation to be used should be identified to the extent possible. The relationships of the information to be gained from complementary biohazard tests, and anticipated problems in testing martian materials in such a fashion should be specified to the degree possible. This Sub-group will recommend methods and concepts to be discussed at a later workshop in support of protocol development."

The Members of Sub-group 6 were:

Hawley, Robert (Chairperson)  
Sourdive, David (Co-Chairperson)  
Candresse, Thierry  
Eisen, Jonathan  
Fishbein, William  
Fultz, Patricia  
Gabriel, Dean  
Gerba, Charles

Granges, Jacques  
Jahrling, Peter  
Khan, Ali  
Kovacs, Gregory  
Leonard, Debra  
Moutou, François  
Murphy, William  
Relman, David  
Richmond, Jonathan  
Tennant, Raymond  
Vasil, Indra  
Viso, Michel

Sub-group 6 identified the need for preliminary characterization of sample material (e.g., color, size, shape, origin, etc.) as part of its baseline information for proceeding with biohazard assessments. As outlined in the flowchart shown in figure 3, a portion of the Mars sample (50-100 g) will be used to complete a comprehensive life detection and biohazard protocol, including chemical and radiological tests to determine if any of these hazards exist.<sup>7</sup> In addition to testing fines and rock samples, the ambient gas phase adjacent to martian samples should also be tested for these hazards, and compared to a sample of martian atmosphere collected and stored in the absence of solid phase sample. The solid sample should also be analyzed by X-ray imaging and 3-dimensional image analysis using the synchrotron for total carbon, structure of carbon chains, and centers of asymmetry. The purpose of the carbon analyses is to determine the most probable location(s) of biological material, if any, within the samples.

Sub-group 6 specifically noted that some tests could be done at locations other than the primary receiving and containment facility as long as maximum containment and security of the sample is maintained (i.e., the sample must be kept completely isolated within multiple containers that are appropriately nested, sealed, and intact). The rationale for being able to test the sample outside of containment is based on the availability of adequate procedures for containing the sample, for sterilizing or cleaning the outside of the sample container, and for returning the sample to the containment facility after non-invasive or non-destructive synchrotron analysis.

Following preliminary characterization and testing, the Mars sample will be tested for the presence of biohazards using *in vitro* and *in vivo* testing protocols. All biohazard testing will be conducted under strict containment at the primary receiving facility or other similarly secure maximum containment facility. For their purposes, this Sub-group defined a biohazard as a substance (material or entity, of biological origin, replicating or non-replicating) capable of producing an adverse effect on a biological system. If hazardous, or capable of producing an effect, the nature of the hazard (e.g., strong chemical oxidizer, radioactive, replicating life form, etc.) must be ascertained so that appropriate subsequent handling procedures can be determined.

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7. If radiological or chemical hazards are detected in the sample, it is assumed that appropriate containment and handling will be required to protect personnel working with the sample materials.

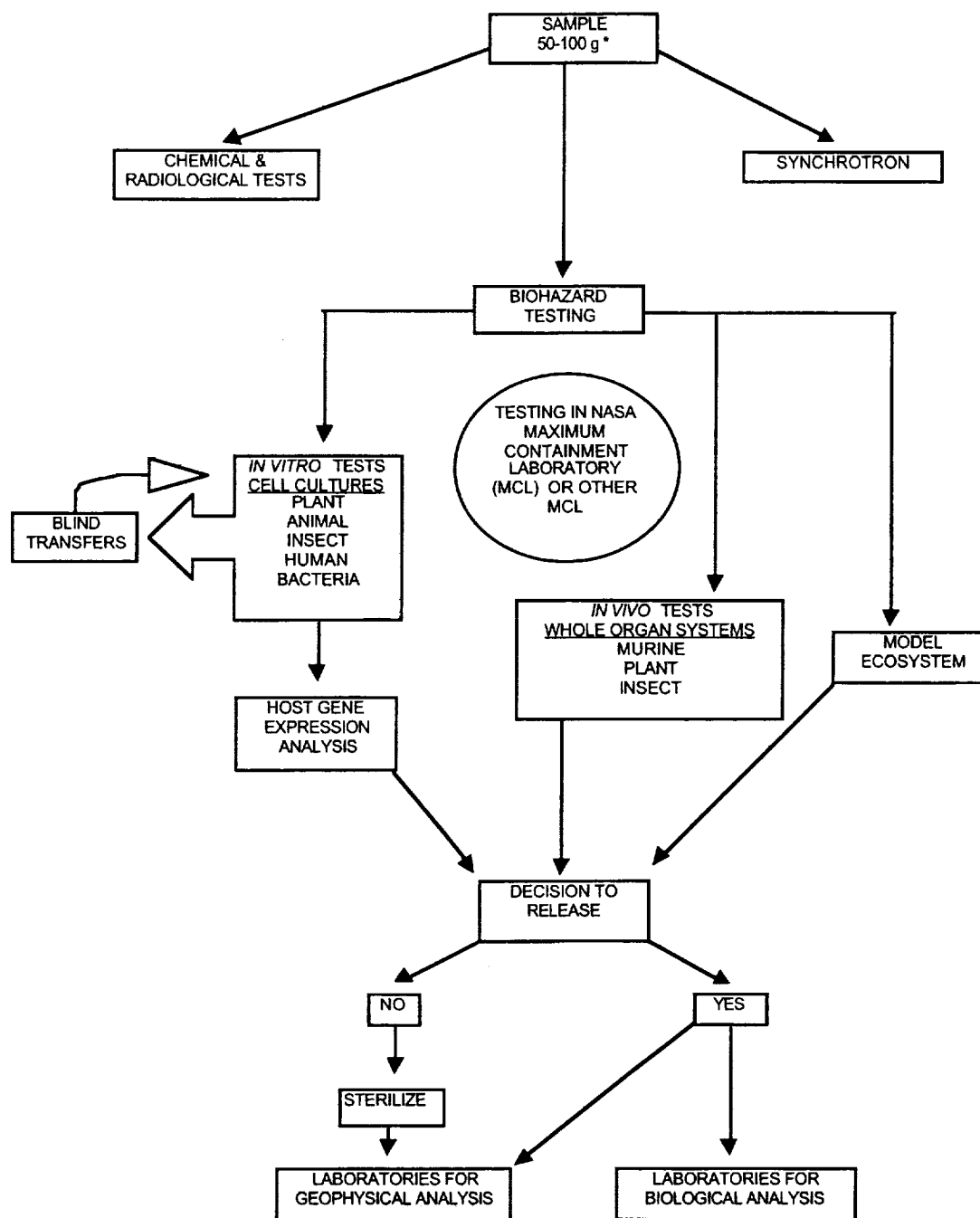


Figure 3: Proposed Biohazard Testing Process

In the process of testing, the sample materials may be ground, crushed, surface-washed, and/or solubilized to optimize sample preparation. Aqueous and solvent-extracted samples should be tested. Representative samples as well as the sample size for each analysis remains to be determined. However, sample size could be determined on a case-by-case basis, based on calculations derived from carbon content. Samples should be subjected to quality control analysis during each procedure, that is, non-treated samples (or irradiated control samples) should also be analyzed to determine if the extraction procedure affects analysis.

Sub-group 6 recommended that *in vitro* testing should employ primary and established cell lines derived from plants, animals, insects, and humans. Bacterial cell cultures should also be tested. If standardized microbial community ecosystem models are available, they should also be tested for their response to martian samples. Supernatant fluids and cellular material should be sub-cultured (blind-transfers) to detect possible replicative properties of any biohazard.

All cell cultures should be analyzed for phenotypic responses (e.g., viability, cytopathic effect, and other morphological changes) using routine procedures, as well as host gene expression responses using high density DNA micro-arrays, and changes in host cell global protein profiles.

For *in vivo* testing, Sub-group 6 recommended the use of mouse, plant, and insect models in an attempt to detect any biohazard in the Mars sample. In the mouse system, knockout mice with broad immune defects should be used to provide an organism system maximally sensitive to biological challenge. After inoculation and various periods of observation, mice should be subjected to thorough gross and microscopic histological examination and tissues tested by DNA micro-array analysis. Specific Pathogen Free (SPF) 'out-bred' mice should also be inoculated with the Mars sample. The *Arabidopsis thaliana* model plant system should also be used, possibly including a mutant *A. thaliana* line with a defective defense system. After inoculation and various periods of observation, plants should be tested by micro-array analysis. Representative plants from at least twelve (12) different plant families should be used to detect any biohazard in the Mars sample. Plants should be inoculated systemically and topically to maximize biohazard detection. Insects from a variety of families and genera should also be used for biohazard detection. A selected model ecosystem should also be used in an attempt to detect any biohazard (e.g., disruption of biogeochemical cycles) in the Mars sample. This Sub-group recommended that all *in vitro* and *in vivo* assays be well-characterized beforehand with terrestrial samples that are felt to best mimic the expected properties of martian samples.

Upon completion of the *in vitro*, *in vivo*, and model ecosystem testing (in combination with information from preliminary characterization and life detection tests), a decision will be made whether or not to release any of the Mars sample. If no biohazard or life form is detected, the Sub-group agreed that sample(s) may be selected for release from maximum containment (BSL-4) (see Note 4, Appendix G). Sub-group 6 suggested that additional experiments should be done in approved facilities under BSL-3 containment principles and practices, including life detection experiments, on a case-by-case basis (see Note 1, Appendix G). Such experiments should be approved by a peer-review panel convened to evaluate proposals, including life-detection proposals, which may require non-irradiated samples for testing.



The Sub-group also agreed that some samples may be released prior to completion of biohazard and life-detection testing. The sample(s) selected for such release should be subjected to extensive gamma ( $\gamma$ ) irradiation for sterilization to eliminate any potential biohazard that failed to be detected in the analysis described above (see Note 5, Appendix G). The dose and duration of  $\gamma$  irradiation should be determined based on available evidence in the literature gleaned from model biological systems. Efficacy studies on the sterilization methods and specifications should be conducted before release of any Mars sample(s) to outside laboratories for geophysical or other analyses. However, it is anticipated that final decisions on sterilization conditions will be based on an understanding that terrestrial models may not adequately represent martian life forms, and that a prudent approach may require more vigorous or destructive conditions than might be indicated by these models. If the decision is reached *not* to release any material because of biohazard concerns, the Sub-group recommended that samples should be retained and analyzed for geophysical properties within the primary receiving and containment facility.

Members of the Sub-group also crafted two alternative decision trees with proposed procedural approaches through the biohazard assessment process. Logic Tree #1 (figure 4) considers the paths for sterilized or un-sterilized samples and leads to decisions about either continued containment or release of samples for curation and subsequent scientific analysis. Logic Tree #2 (figure 5), considers a possible sequencing of analyses leading to release and curation versus continued containment under BSL-4 conditions. Both logic trees are considered preliminary for discussion purposes.

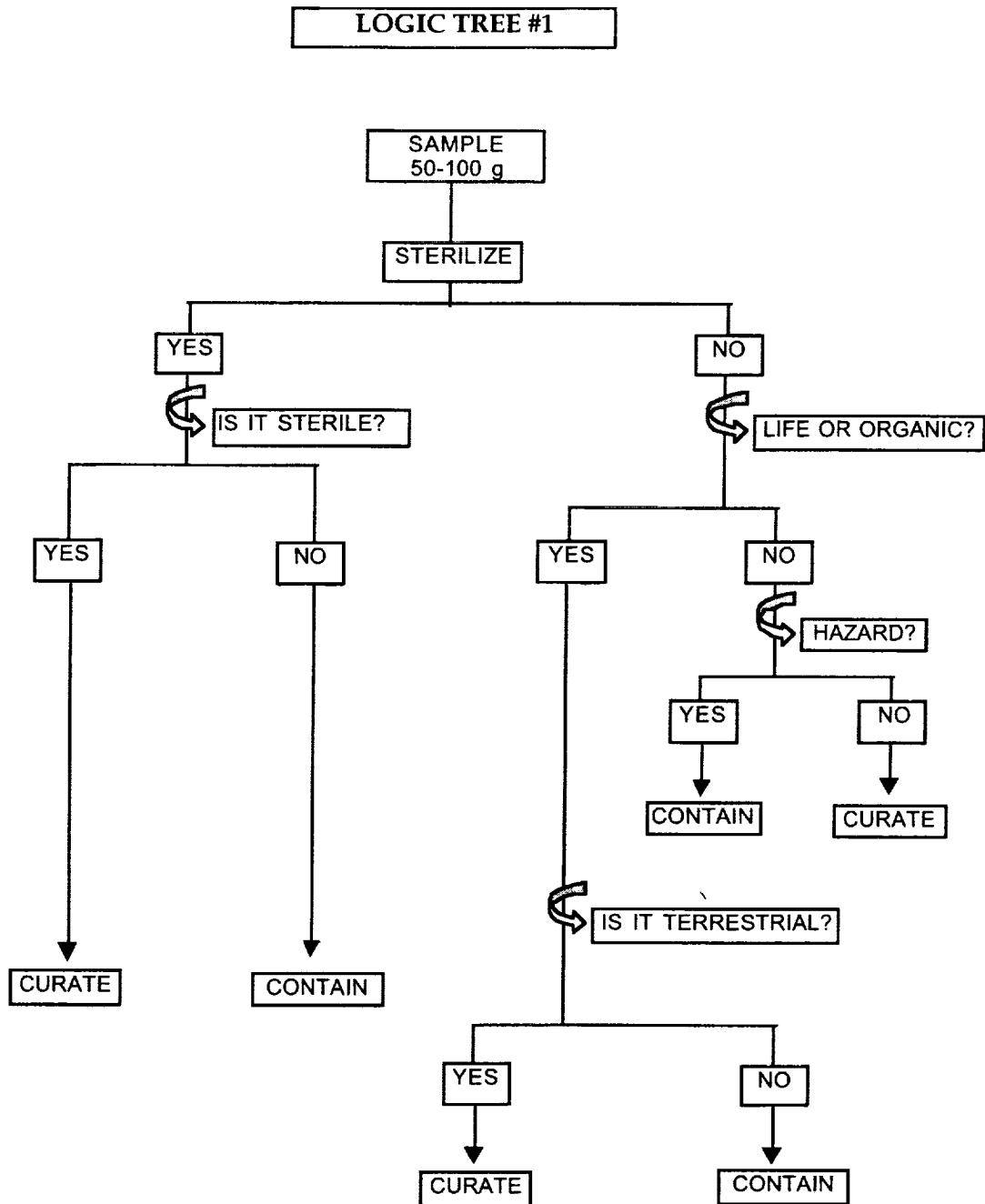


Figure 4 - Logic Tree #1.

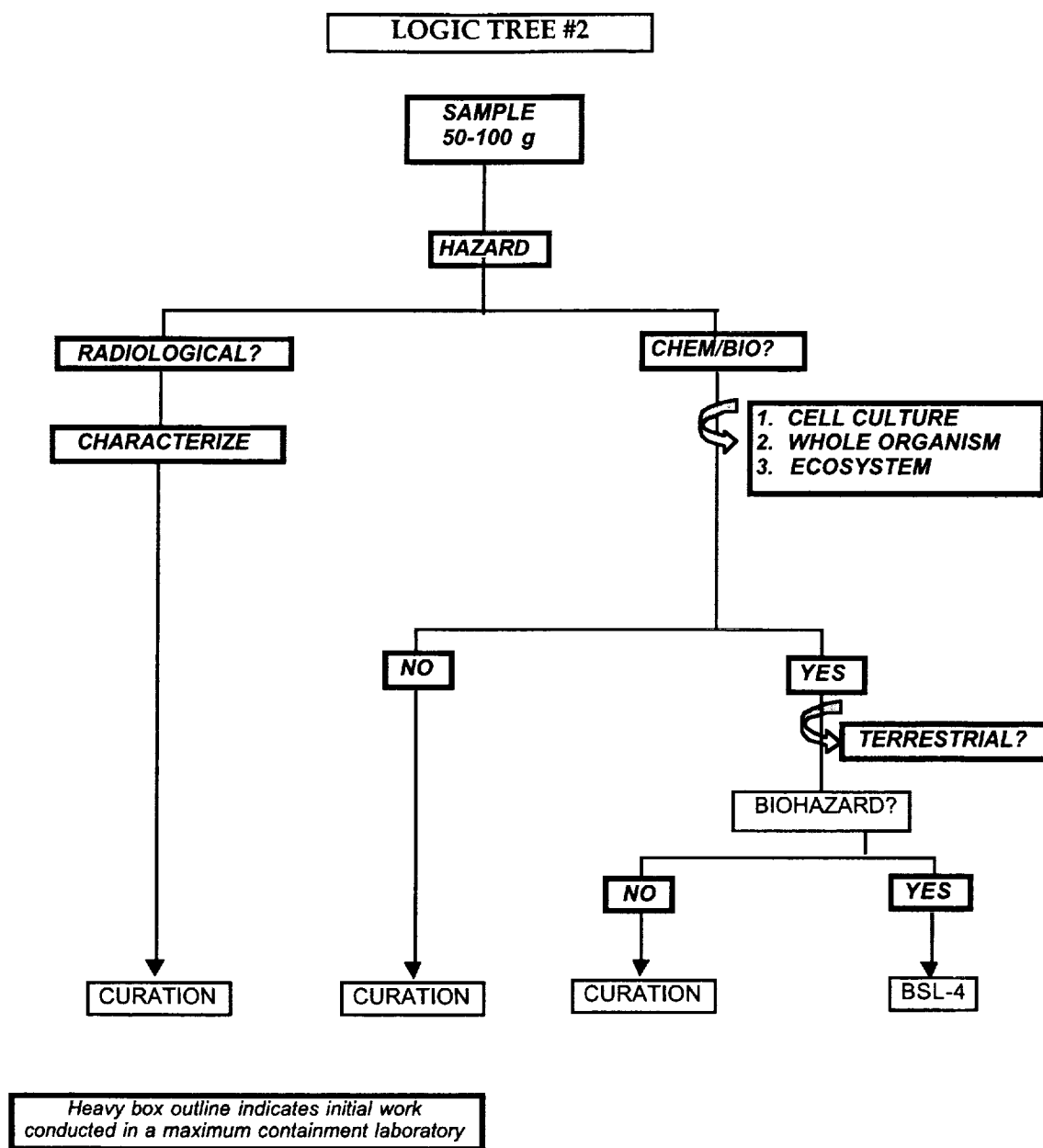


Figure 5 - Logic Tree #2.



## APPENDIX A: WORKSHOP 1 AGENDA

### Day 1 Morning Session (Plenary)

- 8:00 a.m. Welcome and logistics
- 8:10        Organization and Objectives of Workshop Series;  
              Introduction of background lectures for the workshop
- 8:20        Planetary Protection Overview and Mars Architecture Status (J. Rummel)
- 8:35        French Participation in Mars Sample Return (J.-L. Counil)
- 8:45        NRC 1992 and 1997 Reports (K. Nealson)
- 9:10        Mars Sample Return Mission Design (R. Gershman)
- 9:35        Break
- 10:00       Options in Extraterrestrial Sample Handling and Study (D. Papanastassiou)
- 10:25       MSHARP Report (D. DeVincenzi)
- 10:50       Overview of ALH84001 Tests, Equipment, and Interpretation (A. Treiman)
- 11:15       Lunar Sample Protocol (J. Allton)
- 11:30       1997 Quarantine Protocol Workshop Overview (M. Race)
- 12:00       Lunch

### Day 1 Afternoon Session

- 1:00 p.m. Plenary
  - Organization and Objectives of Workshop 1
  - Issues in Protocol Development
    - + Criteria for Release
    - + Context of Collection
    - + Amount of Sample Available
    - + Single/Multiple Containment Facilities
- 1:30        Introduction to Strawman Protocol
- 2:00        Establish three sub-groups to deal with key questions from framework:
  - Preliminary sample characterization requirements
  - Representative sub-samples; nature of sample
  - Sequence of tests; types of testing possible; range of results  
    re: release criteria
- 4:30        Sub-groups report status in plenary session  
              (Sub-group chairpersons assign overnight writing)
- 5:30        Adjourn

**Day 2 Morning Session**

- 8:00 a.m. Day 1 Sub-groups caucus
- 8:30 Day 1 Sub-groups report status in plenary session
- 9:30 Assignments and rationale for forming three sub-groups:
- Physical/Chemical Analyses: methods, sample state, containment, controls
  - Candidate life detection tests: qualifiers, contraindications, controls, characterization
  - Candidate biohazard tests: qualifiers, contraindications, controls, characterization
- 10:00 Break out into three sub-groups
- 12:00 Lunch

**Day 2 Afternoon Session**

- 1:30 p.m. Continuation of three morning sub-groups
- 3:30 Plenary status reports from three Day 2 sub-groups
- 4:00 Plenary Discussion: Quantity of sample required for protocol
- 5:00 Identification of Issues for Day 3 plenary session  
(Day 2 sub-group chairs assign overnight writing)
- 5:30 Adjourn

**Day 3 Morning Session**

- 8:00 a.m. Day 2 sub-groups caucus
- 8:30 Day 2 sub-groups report status in plenary session
- 9:30 Plenary Discussion:
- Criteria for Release
  - Context of Collection
  - Single/Multiple Containment Facilities
- 10:30 Summarize and integrate Workshop 1 results; Identify Open Issues
- 11:30 Develop and discuss draft protocol and identify action items  
Overview of Workshop 2
- 12:30 Adjourn

**Day 3 Afternoon Session**

- Meeting of the Executive Work Group (Planning Committee and Sub-group Chairpersons/Reps)
- 1:30 p.m. Outline Workshop 1 report (Distribute writing assignments and identify Planning Committee action items)
- 4:00 Adjourn

## APPENDIX B1: WORKSHOP 1 PARTICIPANTS' AREA(S) OF EXPERTISE

Name	Affiliation	Area(s) of Expertise
Acevedo, Sara E.	SETI Institute	(Workshop Planning Committee Member)
Allen, Carl	NASA Johnson Space Center	Sample Handling and Curation; Physical/Earth and Planetary Sciences
Allton, Judith H.	NASA Johnson Space Center	Sample Handling and Curation; Physical/Earth and Planetary Sciences
Bibring, Jean-Pierre	IAS, France	Planetology; Sample handling; Curation facility
Bielitzki, Joseph	NASA Ames Research Center	Chief NASA Veterinary Officer
Bogard, Donald	NASA Johnson Space Center	Sample Handling and Curation
Bradley, John	MVA Associates, Norcross GA	Electron Microscopy; Physical/Earth and Planetary Sciences
Candresse, Thierry	French National Institute of Agronomical Research (INRA)	Molecular-based detection and identification techniques for plant viruses and viroids
Chyba, Christopher	Carl Sagan Chair for the Study of Life in the Universe, SETI Institute	Prebiotic Chemistry; Physical/Earth and Planetary Sciences
Counil, Jean-Louis	Centre National d'Etudes Spatiale (CNES)	(Workshop Planning Committee Member)
Crissman, Harry A.	Los Alamos National Lab	Flow Cytology and Cytochemical Life Detection Methods; Life Detection
Cronin, John	Professor, Chemistry and Biochemistry, Arizona State Univ.	Chemistry; Physical/Earth and Planetary Sciences
Debus, André	Centre National d'Etudes Spatiale (CNES)	Mars Sample Return Planetary Protection project manager
Des Marais, David	NASA Ames Research Center	Biogeochemistry; Physical/Earth and Planetary Sciences
DeVincenzi, Donald	NASA Ames Research Center	(Workshop Planning Committee Member)
Dick, Steven J.	US Naval Observatory	Astronomer and Historian of Science
Eisen, Jonathan	Institute for Genomic Research	Radiation resistance and DNA repair, microbial genomics and evolution, characterization of uncultured microbes
Fishbein, William N.	Dept. of Environment and Toxicologic Pathology, Armed Forces Institute of Pathology	Molecular Toxicology; biochemical and molecular pathology; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Flandrois, Jean-Pierre	Centre National de la Recherche Scientifique (CNRS); University of Lyon	Determination of population dynamics models in microbiology for risk assessment and decision-making.

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Friedmann, E. Imre	Florida State University	Microbiology in extreme environments; life detection
Fultz, Patricia N.	Univ. Alabama at Birmingham	Microbiology
Gabriel, Dean W.	Professor, Molecular Plant Pathology, University of Florida	Molecular plant pathology; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Gerba, Charles	Professor, Department of Environmental Microbiology, Arizona State University	Microbial environmental risk assessment
Gershman, Robert	NASA, Jet Propulsion Laboratory	Mars sample return mission design
Granges, Jacques	Laboratoire de Haute Securite P4 Jean Merieux, France	Responsible for the MERIEUX Biosafety Level 4 Facility; experience in biochemical and cancer research and virology
Hawley, Robert	US Army Medical Research Institute of Infectious Diseases, Ft. Detrick MD	Biosafety; emergent biohazard detection and containment methods; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Holland, Heinrich D.	Department of Earth and Planetary Sciences, Harvard University	Earth Sciences
Jahrling, Peter	US Army Medical Research Institute of Infectious Diseases, Ft. Detrick MD	Biosafety, emergent biohazard detection, and containment methods; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Johnson, Dale W.	Desert Research Institute	Soil chemistry; physical/Earth and planetary sciences
Khan, Ali S.	National Center for Infectious Diseases, Centers for Disease Control and Prevention	Biodefense; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Korwek, Edward	Law Offices, Hogan and Hartson	Environmental law and policy
Kovacs, Gregory T.A.	Electrical Engineering, Stanford University	Biodefense; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Leonard, Debra G.B.	Dept. of Pathology and Laboratory Medicine, University of Pennsylvania	Molecular pathology of infectious diseases; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Levinthal, Elliott	Stanford University	Professor Emeritus, School of Engineering
MacPherson, Glenn	Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution	(Workshop Planning Committee Member)
Marty, Bernard	CRPG, France	Isotope geo- and cosmochemistry; primitive Earth mantle geo-dynamics; planetary volatiles including the Moon, Mars, and SNC materials.



Name	Affiliation	Area(s) of Expertise
Maurel, Marie-Christine	Institut Jacques Monod	Microbiology; origin of life
Morowitz, Harold J.	George Mason University, Trustee, The Krasnow Institute, Robinson Professor	Biology and natural philosophy
Moutou, François	Head of the Laboratory of General Epidemiology, Central Laboratory for Veterinary Research	Epidemiology of major animal diseases; modeling of airborne dissemination of the FMD virus; risk analysis methodology and disease control.
Mustin, Christian	Centre de Pédologie Biologique	Geologist and physicochemist; biochemical reactivity of microorganism- mineral interfaces.
Nealson, Kenneth	NASA, Jet Propulsion Laboratory	Post-Viking microbiology/environmental microbiology; life detection
Papanastassiou, Dimitri	NASA, Jet Propulsion Laboratory	Options for Mars sample handling
Pavé, Alain	Laboratoire de Biometrie et de Biologie Evolutive, Université Claude Bernard	Mathematical modeling of living systems. Applications to molecular biology, cellular biology and microbial ecology
Pepper, Ian L.	Professor, Environmental Microbiology, University of Arizona	Soil microbes in arid environments; life detection
Persing, David H.	Corixa Corporation, Seattle WA	Microbial detection methods for unrecognized organisms; life detection
Prieur, Daniel	Station Biologique, University of Brest	Microorganisms under extreme conditions
Prufert-Bebout, Lee	NASA Ames Research Center	(Workshop Planning Committee Member)
Race, Margaret	SETI Institute	(Workshop Planning Committee Member)
Relman, David A.	Dept. of Microbiology and Immunology, Stanford University	Microbial detection methods for unrecognized organisms; life detection
Richmond, Jonathan	Director, Office of Health and Safety, Centers for Disease Control and Prevention	Biosafety, emergent biohazard detection, and containment methods; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Rummel, John	Planetary Protection Officer, NASA Headquarters	(Workshop Planning Committee Member)
Schad, Jack	NASA Headquarters	(Workshop Planning Committee Member)
Sogin, Mitchell L.	Biology and Evolution, Marine Biological Laboratory	Comparative molecular biology and evolution; life detection
Sourdive, David J.D.	Centre d'Etudes du Bouchet	Viral immunology, arenaviruses; High sensitivity detection and identification of potentially hazardous microorganisms
Stabekis, Pericles D.	Lockheed-Martin	(Workshop Planning Committee Member)
Tennant, Raymond E.	National Institute of Environmental Health Sciences, National Institutes of Health	Efficacy of <i>in vitro</i> methods; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis

Name	Affiliation	Area(s) of Expertise
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Vasil, Indra K.	Professor, Plant Cell and Molecular Biology, University of Florida	Plant tissue culture methods and biotechnology; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis
Viso, Michel	Centré National d'Etudes Spatiale (CNES)	Radionuclides in biology, applied medical statistics, animal and comparative immunology, domestic animal nutrition
Wainwright, Norman R.	Senior Scientist, Molecular Biology, Marine Biological Laboratory	Comparative molecular biology and evolution; life detection
Walker, Robert M.	Washington University in St. Louis	Director and Professor, McDonnell Center for the Space Sciences; Department of Physics
Wall, Diana	Colorado State University	Director, Natural Resource Ecology Lab, College of Natural Resources
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## APPENDIX C: SUMMARIES OF KEY PLANETARY PROTECTION REPORTS

In order to give all participants a familiarity of PP issues and history, summaries of key PP reports were provided as pre-workshop reading materials; those summaries are included here. The following reports were summarized:

"Comprehensive Biological Protocol for the Lunar Sample Receiving Laboratory," Baylor University College of Medicine, Manned Spacecraft Center, Houston, Texas, NASA CR9-2209 (1967).

"Overview of Implementation of Quarantine Protocols," J.H. Allton, presentation to Mars Exploration Long Term Science Working Group (MELTSWG), chaired by M. Carr (1997).

"Orbiting Quarantine Facility (OQF); The Antaeus Report," Donald L. DeVincenzi and John R. Bagby, Editors, NASA SP-454, NASA Headquarters, Washington, D.C. (1981).

"Biological Contamination of Mars: Issues and Recommendations," Task Group on Planetary Protection, chaired by Kenneth Nealson, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1992).

"Mars Sample Return: Issues and Recommendations," Task Group on Issues in Sample Return, chaired by Kenneth Nealson, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1997).

"Mars Sample Quarantine Protocol Workshop," D.L. DeVincenzi, J. Bagby, M. Race, and J.D. Rummel, Editors, NASA Ames Research Center, Moffett Field, California, April 1997, NASA CP-1999-208772 (1999).

"Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies," Task Group on Sample Return from Small Solar System Bodies, chaired by Leslie Orgel, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1998).

"Mars Sample Handling and Requirements Panel (MSHARP) Final Report," Michael H. Carr, et. al., NASA, Jet Propulsion Lab, Pasadena California, NASA TM-1999-209145 (1999).

"Size Limits of Very Small Microorganisms: Proceedings of a Workshop," Steering Group for the Workshop on Size Limits of Very Small Microorganisms, A. Knoll and M.J. Osborn, Co-Chairpersons, Space Studies Board, National Research Council, National Academy Press, Washington D.C. (1999).

"Current State of Controversy About Traces of Ancient Martian Life in Meteorite ALH84001," Allan H. Treiman, Lunar and Planetary Institute, Houston Texas (Feb. 2000).

**Summary of: "Comprehensive Biological Protocol for the Lunar Sample Receiving Laboratory," Baylor University College of Medicine, Manned Spacecraft Center, Houston, Texas, NASA CR9-2209 (1967).**

Reason Written

"... a biological quarantine protocol for the safe handling and study of lunar material to be returned to Earth from early Apollo missions ..." The goals of the Lunar Receiving Laboratory were multiple and broadly encompass the scientific disciplines of geology, geophysics, chemistry and biology. The purpose of the protocol was to define the biological studies that might reasonably fulfill the goals of the Bioscience Working Group (NASA-SP-88, p. 234, July 1965). The goals were " ... to provide a formal mechanisms for testing appropriate representative lunar samples for the possible presence of agents that might be infectious or toxic to man, animals, and plants." It should be the goal "... to provide safety clearance for lunar samples, if possible, within a period of approximately 30 days."

The protocol attempted to explore in depth the effect of lunar material upon plants and animal species about which a great deal was already known. The protocol was designed to be flexible and lent itself to easy revision as more information is accumulated concerning the lunar sample and as biological techniques improved during the implementation of the laboratory. The work of the laboratory and protocols was aimed at short-term, time-critical, analytical procedures and identification of whether or not the returned sample constituted a threat to Earth's biosphere. All other considerations became secondary.

The biological protocol has three main elements:

1. Crew microbiology (comparisons with pre-flight microbiology profiles and review of alterations in flora following return to Earth) ... conducted under quarantine and limited in duration to the time required to establish the nature of the microbial burden carried by the crew and the assurance of their freedom from communicable disease.
2. *In vitro* attempts to culture microorganisms from the lunar samples;
3. Direct challenge of the lunar sample in biological systems.

"Acknowledges that ... it will be impossible to tests lunar sample on all but a few Earth species -- so portions will be tested in representative members of all major taxa ... utilizes the concept of 'unity within diversity' and the careful selection of certain key species to provide a broad-based spectrum for testing purposes."

Ways that samples may be injurious to organisms from Earth are from inherent toxicity of material or the capability of the material to propagate itself in Earth species.

Toxic materials were classified as follows:

1. Radioactive
2. Unknown inorganic polymer(s) possibly containing silica, boron, and other inorganic elements
3. Deleterious low-molecular-weight compounds acting as cellular and metabolic poisons, mutagens, irritants, anti-metabolites or anti-vitamins
4. Unknown metallo-organic compounds, effects on terrestrial organisms unknown

Replicative materials were classified as follows:

1. Organisms (viral, bacterial, fungal) taken to the moon and returned in mutated form
2. Plant materials of lunar origin capable of reproducing on Earth as autotrophs, heterotrophs in nutrient media – resulting in naturalized forms producing deleterious effects by contact or competition
3. Xerophilic life forms of lunar origin using as protoplasmic materials elements found in terrestrial organisms such as carbon, hydrogen, oxygen, sulfur and phosphorous.
4. The existence of living matter on the moon at an organizational level above that of small metazoa or metaphytes ... excluded from consideration because probability considerably less than that for unicellular organisms.

Additional items addressed:

1. Philosophy of testing process itself:
  - Requirement for high professional standards in the conduct of studies
  - NASA should avail itself of technical competence existing in laboratories throughout the country
  - Employ outside consultation at all steps
  - Require a high degree of supervision and insight
  - Laboratory management to utilize fullest sound, competent advice of the academic community and relevant federal agencies (Dept. of the Interior, USDA, US Public Health Service)
2. The nature of the internal controls to be employed
3. The statistical approach to an evaluation of a heterogeneous, unknown mixture whose toxic or microbiological potential is unknown (assumes lunar sample, if it contains microorganisms at all, contains them at very low concentrations. Thus assume at either 'near negligible' or at 'detectable' levels – leading to estimate of high and low quantity of material to be employed in challenges.)

Other important points discussed:

1. Sequence of events in handling samples
2. Collection, transport, receipt, opening as well as mixing, aliquoting, and distribution are part of the general protocol
3. Series of challenges to host organisms with both *in vivo* challenges and *in vitro* studies on selected representative plants and animal hosts using classic microbiologic techniques AND parallel studies with both animal and plant cells in tissue culture – all these 'observational' steps to be followed by a secondary *in vivo*-challenge as well as *in vitro* classic microbiological techniques using organic and inorganic media containing such added nutrients as might be suggested by the initial elemental and organic analysis of the lunar sample. This temporal order of initial, followed by secondary challenges, constitutes the critical part of the microbial protocol (emphasis added). If replicating forms exist, this sequence offers the greatest promise for their detection.
4. Every system described in the protocol has as an internal control the requirement that direct challenge of *in vivo* systems be conducted with both untreated and sterilized lunar material under absolute double-barrier techniques.
5. Carefully controlled trial runs of all systems should begin fully one year in advance of receipt of the first lunar samples, and 'unknown' terrestrial soil samples should be carried through all systems to insure the technical competence of the laboratory facility.

**"Overview of Implementation of Quarantine Protocols," J.H. Allton, presentation to Mars Exploration Long Term Science Working Group (MELTSWG), chaired by M. Carr (1997).**

Protocols carried out in Class III biological cabinetry operated under negative pressure and behind the secondary barrier included:

1. Direct observation in which lunar material was examined in native state and via washings and sediments with various optical and electron microscopes up to 1000X magnification
2. Bacteriology/mycology protocols – lunar sample distributed on un-enriched and enriched culture media at temperatures ranging from 4°C to 55°C under cover gases supporting aerobes, micro-aerobes and anaerobes. Prepared lunar sample was tested to support growth of several pathogenic organisms.
3. Virology and mycoplasma protocols for toxic effects in which tissue cultures of African green monkey (GMK), human embryonic kidney (HEK), and human embryonic lung (HEL) tissues were challenged with lunar sample. For virus isolation, embryonic chicken eggs and 6 tissue cultures (HEK, GMK, HEL, primary duck fibroblast, heteroploid bovine kidney, and heteroploid porcine kidney) were challenged with lunar material. Poikilothermic animals such as trout, minnow, and grunt fin, and 3 mycoplasma media were exposed to lunar material.
4. Mammalian protocols in which mice (180) were injected with lunar material and cultures and tissue samples were taken from sacrificed animals.
5. Avian protocols in which finely powdered suspension injected intraperitoneally into 90 Japanese quail.
6. Invertebrate and fish protocols in which lunar material was added to food for terrestrial and to water for aquatic animals. Test organisms were paramecium, planaria, oyster, cockroach, house fly, wax moth, brown shrimp, killifish, guppy, and minnow.
7. Botany protocols in which assessments were made of lunar sample effects on reproduction and morphology of algae, germination and development of spores and seeds, growth of seedlings, growth and differentiation in tissue cultures. Thirty-five species used including algae, onion, tobacco, radish, spinach, cotton, tomato, potato, wheat, bean, etc. (mostly food crops).
8. Each class of protocol had a decision tree for quarantine testing or sample release recommendations, but all were similar: If any differences between exposed group and control occurred that were not explained as terrestrial contamination, then second order testing was recommended; otherwise release of samples was recommended. No evidence of replicating agents was found in the test systems used, and all samples were released unconditionally.

**Summary of: "Orbiting Quarantine Facility (OQF); The Antaeus Report," Donald L. DeVincenzi and John R. Bagby, Editors, NASA SP-454, NASA, Washington, D.C. (1981).**

**Charter**

A NASA design study was conducted in 1978 to examine the feasibility of designing, constructing, and operating a unique space-based laboratory – one dedicated, at least initially, to the isolation and analysis of potentially hazardous samples returned from Mars. This report does not argue that analysis of Mars samples should be done in space. Rather, it defines the characteristics of an orbiting laboratory should this be an option for active consideration for future MSR studies. Hence, a considerable effort was devoted to development of an appropriate series of tests to be performed on the sample (the 'quarantine protocol') and to design of the



facility in which these tests would be conducted. The 10-week summer study involving twenty (20) scientists and engineers was intended to be an intensive learning experience for the participants.

### Background

As a result of the Viking missions to Mars, a great deal of knowledge was gained about the surface features and composition the planet. However, one of the major questions that prompted the mission – Is there life on Mars? – was not conclusively answered. Because of that uncertainty, many scientists believed that the samples should be considered to be potentially hazardous until proven conclusively that they are not. This meant that adequate precautions need to be taken to protect the Earth's biosphere until the samples are proved safe. Previously, consideration had been given to returning a sterilized sample. Alternatively, it had been suggested that the sample be held under quarantine in a maximum containment facility on Earth, possibly in a remote location, while undergoing analysis. No one had studied a third option, which was to perform hazard analysis of the sample before it was introduced into the terrestrial biosphere. Therefore, this summer study was convened in 1978 to examine the feasibility of receiving and analyzing returned Mars samples in an orbiting quarantine facility.

### Summary and Conclusions

**Mission objective:** The purpose of the Orbiting Quarantine Facility (OQF) would be to detect the presence of biologically active agents – either life forms or uncontrolled (replicating) toxins – in the sample and to assess their potential impact on terrestrial systems. Only when the sample could be certified safe or controllable would it be transferred to laboratories on Earth for physical analysis.

The particular advantage of an orbiting facility over an Earth-based one is the flexibility it offers in the event that potentially pathogenic agents are present in the sample. With space as a buffer between such organisms and the terrestrial biosphere, the risk of terrestrial contamination is far lower. Complete characterization of the hazard such organisms might represent could thus be carried out without fear of a containment failure and possible contamination of the biosphere. Depending upon the results of testing, the options available for subsequent disposition of the sample would include: 1) unqualified release, 2) sterilization prior to release to Earth laboratories, 3) indefinite retention in orbit for prolonged study, and 4) in one extreme case, boosting the sample-containing facility into a distant orbit. A terrestrial quarantine facility could not offer such margins of security.

**Mission scenario:** The mission plan calls for the Space Shuttle to deliver the OQF, one or more components at a time, into near Earth orbit, where it will be assembled and manned. While awaiting the arrival of the Mars Sample Return Vehicle (MSRV), the crew will conduct system tests and protocol review. The incoming MSRV, bearing the sample in a sealed canister in its crown, will be inserted into the same orbit in the vicinity of the OQF. An orbiting transfer vehicle comprised of an Inertial Upper Stage Engine (IUS) and Remote-Teleoperated-Manipulator System (TELLE) will then link up with the MSRV, extract the sample canister, and deliver it to the OQF. Re-supply of the laboratory, replacement of crewmembers if necessary and eventual transport of the sample and crew to Earth will all be carried out via the Space Shuttle.

**Modules:** The proposed facility will consist of five Spacelab-derived modular units, each dedicated to a specific function or group of functions. The overall OQF will be free flying and will have a pinwheel configuration, with four of the cylindrical modules connected spoke-fashion to a central hub. Such a design produces low aerodynamic drag and is easy to assemble; it also allows efficient inter-module movement.

Central to the OQF mission is the Laboratory Module, in which the quarantine testing protocol will be carried out. This unit is equipped with a centrally located containment cabinet system for sample handling and processing. To obtain greater containment reliability than is offered by rubber gloves, specially designed metal bellows manipulative arms will be employed for access to the cabinets. Provision is made to maintain portions of the cabinetry under simulated martian environmental conditions, and a variety of other controlled environments required by the protocol can be produced. Clean air is continuously passed down the face of the cabinets, which are kept under negative pressure to eliminate leakage into the laboratory.

The high-hazard containment facility at the Center for Disease Control (CDC) served as a model for design of many of the physical features and procedures employed in the Laboratory Module. Based on CDC practices, the module itself acts as a barrier to contamination. All equipment and materials leaving the laboratory must be sterilized and packaged in leak-proof containers. Personnel entering or leaving the module must pass through a decontamination area, where they disrobe and take an air shower. The laboratory has independent life support, waste storage, and air filtration systems, and its atmospheric pressure is slightly lower than that of the other modules – all features that ensure effective containment. It is fully equipped for the performance of the quarantine protocol. A variety of microscopes, including scanning electron microscope, are provided. Cameras, spectrophotometers, centrifuge and vacuum devices, autoclaves, refrigerators, and all other necessary laboratory equipment and instruments are present as well.

Four other modules comprise the OQF. The Habitation Module is the crew's living quarters. The OQF's source of power is the Power Module. A general purpose Logistics Module provides storage for supplies and for waste materials generated in the Habitation Module (the Laboratory Module has independent waste storage). A Docking Module, serves as a common interface linking the other four.

**Personnel:** The crew would probably consist of five members: a commander (an astronaut/engineer) and four scientists (a medical doctor, a geobiologist, a biochemist, and a general biologist). Their tasks would be of two general types: facility operation and maintenance, and laboratory work. The allocation of functions and the scheduling of activities have been carefully worked out for each crewmember.

**Experimental protocol:** A number of factors impact the experimental design. For example, the protocol must take into account the limited amount of sample available for testing (probably about 100 g). In addition, it must ensure that the untested portion of the sample remains unaltered. It must include a sufficient range of tests to allow biologically active agents to be detected with a high degree of confidence. Equipment and experiments alike must be appropriate for use in the zero-g environment. The potential for human error must be minimal.

And there must be enough flexibility designed into the protocol to permit a thorough characterization of life forms that might not closely resemble terrestrial forms.

**Preliminary handling:** The protocol begins with receipt of the sample canister from the IUS-TELLE. A collapsible structure in the OQF guides the transfer vehicle into position so that a trigger mechanism and clamp can acquire the canister and draw it into the OQF's airlock. The sample canister is punctured with a needle and a sample of the gas within the canister is taken. A mechanism similar to a can opener then removes the bottom of the canister so that further gas sampling and removal of a sub-sample can take place. The sub-sample, consisting of approximately 100 g (or ~10 percent) of the returned sample, is first analyzed for radioactivity and then transferred by a manipulator to a sample processing unit.

This unit is specially designed to permit the sub-sample to be manipulated in the absence of gravity, by means of centrifugal force. In the processing unit, the sample is sized and larger particles are viewed under a stereomicroscope to determine whether organisms or fossils are present. The larger-sized material is then evenly ground and the entire sub-sample is recombined and mixed. This mixture is dispensed to the five testing phases. Of the 100-g sub-sample, 46 g will be used in the various tests; 54 g will be held in reserve for possible further series of tests. The remaining 900 g of sample material is stored, unopened, under martian environmental conditions for later delivery to Earth (if approved).

**Testing protocol:** The five testing phases, and the specific experiments they include, are:

1. Chemical analysis
  - pH, Eh, and conductance tests
  - aqueous extraction/element analysis
  - organic mass spectrometry
  - amino acid analysis
2. Microscopy
  - stereomicroscopic examination
  - scanning electron microscopy
  - light microscope examination
  - ultraviolet microscopy
3. Metabolic testing
  - gas exchange: dry
  - CO<sub>2</sub> fixation: dry and moist
  - enriched O<sub>2</sub> metabolism
  - autoradiography of labeled samples
4. Microbiological culturing
  - growth on solid media
5. Challenge culture

The challenge culture phase involves the introduction of martian soil into cell cultures representing a cross section of terrestrial species. Although a number of organisms have already been tested in zero g to date, additional research is necessary to determine the most appropriate species to include in the challenge system. Such organisms must not only be representative of the Earth's major phyla, but must also have a minimal reaction to zero g.

If results of the preceding series of tests show no evidence of non-terrestrial life forms or replicating toxins, the sample will be approved for delivery to Earth, where more extensive physical, chemical, and biological studies will be undertaken. However, in the event that biological agents have been detected, second order tests would be initiated. The precise character of second-order testing cannot be established in advance. The type of tests would be determined on the basis of characteristics such organisms or toxins might possess.

**Protocol planning:** The protocol is a complex network of interdependent tests, with many activities being dependent upon the outcome of previous tests. To illustrate the sequence of events in the protocol, a tracking technique known as Graphical Evaluation and Review Technique (GERT) is used. GERT charts present test activities and information flows in their proper sequence, and use GERT 'symbolology' to indicate the logic that determines each protocol step. By this means, it is possible to calculate the probabilities associated with different experimental outcomes, and thus to calculate the detection sensitivity of various tests. Detailed GERT charts are presented for each testing phase, along with tables of associated outcome probability analyses.

### Conclusion

The facility and the experimental protocol described here offer a strong margin of protection against the possibility that a Mars sample would contain hazardous agents. They also offer a powerful hedge against the unknown, and against the fears that could easily develop if organisms showing signs of pathogenicity were detected in a sample undergoing study in a laboratory on Earth. With such a sample held in orbit, its disposition could be determined on the basis of analysis rather than emotion, and the scientific value of the returned sample could thus be maximized.

**Summary of: "Biological Contamination of Mars: Issues and Recommendations," Task Group on Planetary Protection, chaired by Kenneth Nealson, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1992).<sup>8</sup>**

### Reason Written

In anticipation of planned robotic missions to Mars in the early 1990's by both the U.S. and Russia, NASA requested advice from the SSB on how to update the nature of planetary protection requirements to reflect changes in the years since the Apollo and Viking missions, and to incorporate new thoughts about life on Mars and the growing environmental awareness of the populace. Recommendations were requested in time for the 1992 COSPAR meeting in order to update international planetary protection policies as needed.

### Background

The Task Group focused on making recommendations concerning the protection of Mars from forward contamination (i.e., contamination of the martian environment by terrestrial organisms) during upcoming missions. It specifically considered then-current views about the chemical and physical properties of Mars, as well as the potential survival of terrestrial organisms on Mars, and the approaches to planetary protection used by the U.S. and Russia. In its deliberations,

8. Available on line: [www.nas.edu/ssb/ssb.html](http://www.nas.edu/ssb/ssb.html) (then select 'Reports' and '1992').

the task group distinguished between missions whose goals included reconnaissance and measurement vs. those that specifically included experiments to detect life.

### Findings

The task group viewed the problem of forward contamination as separable into two principal issues: 1) the potential for growth of terrestrial organisms on Mars ( $P_g$ ), and 2) the importation of terrestrial organic contaminants, living or dead, in amounts sufficient to compromise the search for evidence of past or present life on Mars itself.

1. Based on current knowledge of conditions on Earth that limit cell growth and on the best estimates of surface conditions on Mars, the task group concludes that no known terrestrial organisms could grow on the martian surface. However, this fact does not alter the case as far as contamination of a possible past or extant martian biosphere is concerned. Prudence dictates that bio-load reduction on all lander missions to Mars must continue to be seriously addressed. The issue of spacecraft cleanliness is particularly crucial when life-detection experiments are included in the scientific payload.

The task group concurred unanimously that "Forward-contamination, solely defined as contamination of the martian environment by growth of terrestrial organisms that have potential for growth on Mars, is not a significant hazard. However, forward-contamination more broadly defined to include contamination by terrestrial organic matter associated with intact cells or cell components is a significant threat to interpretation of results of *in situ* experiments specifically designed to search for evidence of extant or fossil martian microorganisms."

2. Advances in techniques for assessing the existence of microorganisms will have a strong impact both on bioburden assessment procedures and on future life-detection experiments because of their increasingly greater sensitivity and specificity. The task group strongly recommends that efforts be made to explore current analytical methods for use in bioburden assessment and inventory procedures before spacecraft assembly and launch. Specific promising methods identified included epifluorescent microscopic techniques for directly counting viable cells, and the polymerase chain reaction which increases detection sensitivity by enzymatically amplifying specific biomarkers of even a single cell to detectable levels.

### Recommendations for control of forward-contamination:

1. Landers carrying instrumentation for *in situ* investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined, with sterilization requirements driven by the nature and sensitivity of the particular experiments. The objective of this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter and/or microorganisms deposited at the landing site.
2. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level pre-sterilization procedures – such as clean-room assembly and cleaning of all components – for bio-load reduction, but such spacecraft need not be sterilized.
3. The task group emphasizes that the philosophical intent underlying the 1978 report – to protect Mars from terrestrial contamination so as not to jeopardize future experiments aimed at detecting martian life – is still profoundly important.

### Additional Recommendations:

1. Research: The task group strongly recommends that a sequence of un-piloted missions to Mars be undertaken well in advance of a piloted mission. With regard to these

missions, the task group recommends that a broad spectrum of martian sites be examined, with emphasis on measurements that provide data most likely to contribute to models that provide for a better understanding of the probability of life on Mars and where best to go to find it.

2. **Assessment of Spacecraft Bio-Load:** The task group's recommendation to reduce bio-load on all spacecraft and to sterilize those spacecraft used in life-detection missions assumes the use of Viking procedures. However, the task group recommends that the Viking protocols for assessment of spacecraft bio-loads be upgraded to include state-of-the-art methods for the determination of bio-load. It is critical that methods for assessing bio-load be compatible with methods used to detect life, with methods for both assessment and detection reflecting the same limits and sensitivity. ... modern methods of bioburden assessment should be developed for and applied to spacecraft destined for future Mars missions, especially those carrying *in situ* extant life-detection experiments. ... the development of the methodology in anticipation of future life-detection missions is absolutely essential.

#### Other Issues:

1. **Piloted Versus Un-Piloted Missions:** Missions carrying humans to Mars will contaminate the planet. It is therefore critical that every attempt be made to obtain evidence of past and/or present life on Mars well before these missions occur.
2. **Societal Issues:** A substantial number of active national and international organizations are on the alert for environmental abuse. There is every reason to take seriously the concern (already expressed in some cases) about contamination of Mars and almost certainly about the issue of back-contamination of Earth by martian samples. ... the task group recommends that NASA inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return.
3. **Legal Issues:** There are also legal issues that must be addressed, involving international restrictions as well as federal, state, and local statutes that may come into play. There are currently no binding international agreements concerning forward or back-contamination. The task group recommends as essential that efforts be made: 1) to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration, and 2) to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars. Furthermore, the task group recommends that NASA make a strong effort to obtain international agreement for a planetary protection policy.
4. **NASA Planetary Protection Program:** Although a planetary protection officer currently exists at NASA, there is no budgeted program (as there was during the Viking Program) to implement needed planetary protection research, a public education program, examination of legal and international issues, and the like. The task group recommends that NASA redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the recommendations made in this report.

#### Summary of Recommendations

All of the recommendations put forward by the task group in this report are summarized below. Each is discussed further in the full report in the chapter(s) indicated.

1. Efforts should be made to adopt current molecular analytical methods for use in bioburden assessment and inventory procedures for spacecraft assembly and launch for future missions, and also to develop new methods for the same purposes (Chapters 4 and 5).
2. Landers carrying instrumentation for *in situ* investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined; Viking technology may be adequate, but requirements will

undoubtedly be driven by the nature and sensitivity of the particular experiments. The rationale for this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter that is deposited at the site by microorganisms or organic residues carried on the spacecraft (Chapter 5).

3. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level pre-sterilization procedures (such as clean-room assembly and cleaning of all components), for bio-load reduction, but such spacecraft need not be sterilized (Chapter 5).
4. A sequence of un-piloted missions to Mars should be undertaken well in advance of a piloted mission (Chapter 6).
5. A broad spectrum of martian sites should be examined with emphasis on measurements that provide data most likely to contribute to a better understanding of the probability of life on Mars and where best to go to be able to detect it (Chapter 6).
6. The Viking protocols for assessment of spacecraft bio-loads should be upgraded to include state-of-the-art methods for the determination of bio-load (Chapter 6).
7. NASA should inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return (Chapter 6).
8. It is essential to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars (Chapter 6).
9. NASA should make a strong effort to obtain international agreement for a planetary protection policy (Chapter 6).
10. NASA should redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the above recommendations (Chapter 6).

**Summary of: "Mars Sample Return: Issues and Recommendations," Task Group on Issues in Sample Return, chaired by Kenneth Nealson, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1997).<sup>9</sup>**

#### Reason Written

As stated in NASA Management Instruction 8020.7, the Space Studies Board (SSB) of the National Research Council (NRC) serves as the primary adviser to NASA on planetary protection policy, the purpose of which is to preserve conditions for future biological and organic exploration of planets and other solar system objects and to protect Earth and its biosphere from potential extraterrestrial sources of contamination. In October 1995 NASA requested that the SSB examine and provide advice on planetary protection issues related to possible sample return missions from Mars and other near-Earth solar system bodies. In response, the Space Studies Board established the Task Group on Issues in Sample Return to address the following concerns:

1. The potential for a living entity to be included in a sample to be returned from another solar system body, in particular Mars;
2. The scientific investigations that should be conducted to reduce uncertainty in the above assessment;

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9. Available on line: [www.nas.edu/ssb/mrsrmenu.html](http://www.nas.edu/ssb/mrsrmenu.html)

3. The potential for large-scale effects on the environment resulting from the release of any returned entity;
4. The status of technological measures that could be taken on a mission to prevent the unintended release of a returned sample into Earth's biosphere; and
5. Criteria for controlled distribution of sample material, taking note of the anticipated regulatory framework.

Although focused on sample return missions from Mars, the recommendations can be generalized to any mission that could return a sample from an extraterrestrial object with a similar potential for harboring life.

### Findings

1. Although current evidence suggests that the surface of Mars is inimical to life as we know it, there remain plausible scenarios for extant microbial life on Mars – for instance in possible hydrothermal oases or in subsurface regions.

The surface environment of Mars, from which early samples are most likely to be returned, is highly oxidizing, is exposed to a high flux of ultraviolet radiation, is devoid of organic matter, and is largely devoid of liquid water. It is unlikely that life of any kind, as we currently understand it, either active or dormant, could survive in such an inhospitable environment. If active volcanism, or near-surface liquid water, is discovered on Mars, or if the subsurface environment is found to be considerably less oxidizing and wetter than the surface, the occurrence of extant life on the planet becomes more plausible.

2. Contamination of Earth by putative martian microorganisms is unlikely to pose a risk of significant ecological impact or other significant harmful effects. The risk is not zero, however.

In the event that living martian organisms were somehow introduced into Earth's environment, the likelihood that they could survive and grow and produce harmful effects is judged to be low. Any extant martian microorganisms introduced into Earth's biosphere would likely be subject to the same physical and chemical constraints on their metabolic processes as are terrestrial organisms. Thus, extraterrestrial organisms would be unlikely to mediate any geochemical reactions that are not already catalyzed by Earth organisms. They would be unlikely to be able to compete successfully with Earth organisms, which are well adapted to their habitats.

Because pathogenesis requires specific adaptations to overcome the extensive defenses possessed by all Earth organisms, virulent extraterrestrial pathogens are unlikely. Subcellular disease agents, such as viruses and prions, are biologically part of their host organisms, and so an extraterrestrial source is extremely unlikely. Conceivably, putative extraterrestrial organisms could be capable of opportunistic infections or toxicity, as are some terrestrial bacteria, but such a risk can be eliminated by standard laboratory control procedures.

The potential for large-scale effects, either through pathogenesis or ecological disruption, is extremely small. Thus, the risks associated with inadvertent introduction of exogenous microbes into the terrestrial environment are judged to be low. However, any assessment of the potential for harmful effects involves many uncertainties, and the risk is not zero.

3. Uncertainties with regard to the possibility of extant martian life can be reduced through a program of research and exploration that might include data acquisition from orbital platforms, robotic exploration of the surface of Mars, the study of martian meteorites, the study of Mars-like or other extreme environments on Earth, and the study of returned samples. However, each returned sample should be assumed to contain viable exogenous biological entities until proven otherwise.



The Space Studies Board task group strongly endorses NASA's Exobiological Strategy for Mars Exploration [NASA 1995]. Such an exploration program, while likely to greatly enhance our understanding of Mars and its potential for harboring life, nonetheless is not likely to significantly reduce uncertainty as to whether any particular returned sample might include a viable exogenous biological entity – at least not to the extent that planetary protection measures could be relaxed.

#### Recommendations – Sample Return and Control

1. Samples returned from Mars by spacecraft should be contained<sup>10</sup> and treated as though potentially hazardous until proven otherwise. No un-contained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.

While the probability of returning a replicating biological entity in a sample from Mars, especially from sample return missions that do not specifically target sites identified as possible oases<sup>11</sup> is judged to be low and the risk of pathogenic or ecological effects is lower still, the risk is not zero. Therefore, it is reasonable that NASA adopt a prudent approach, erring on the side of caution and safety.

2. If sample containment cannot be verified en route to Earth, the sample, and any spacecraft components that may have been exposed to the sample, should either be sterilized in space or not returned to Earth.

The engineering and design of any sample return mission should incorporate some means of verifying sample containment during transit and prior to return to Earth. Means should also be available to sterilize the sample, and any spacecraft components that may have been exposed to it, in flight or to prevent their return to Earth in the event that containment cannot be verified.

3. Integrity of containment should be maintained through reentry of the spacecraft and transfer of the sample to an appropriate receiving facility.

The points in a mission where loss of containment is most likely to occur include operations on the martian surface; inter-vehicle transfer of sample material; vehicle reentry, descent, and landing; and subsequent transfer of the sample container to a receiving facility. Techniques and protocols that can ensure containment at these vulnerable points should be designed into the mission.

4. Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized.

Returned samples should be considered potentially hazardous until they have been reasonably demonstrated to be non-hazardous. Distribution of unsterilized sample material should occur only after rigorous physical, chemical, and biological analyses confirm that there is no indication of the presence of any exogenous biological entity. If any portion of the sample is removed from containment prior to this determination, it should first be sterilized. The development of effective sterilization techniques that preserve the value of treated material for other (non-biological) types of scientific analysis should be the subject of research by NASA and by the science team associated with the sample-receiving facility.

5. The planetary protection measures adopted for the first Mars sample return missions should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body.

Samples returned from the martian surface, unless returned from sites specifically targeted as possible oases, are unlikely to harbor life as we know it, and there may be

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10. The words 'contained' and 'containment' are used herein to indicate physical and biological isolation.

11. Locations that exhibit active volcanism or where the presence of liquid water is indicated.

some pressure to reduce planetary protection requirements on subsequent sample return missions if prior samples are found to be sterile. Presumably, however, subsequent missions will be directed toward locations on Mars where extant life is more plausible, based on data acquired from an integrated exploration program, including prior sample return missions. Thus, planetary protection measures may become more rather than less critical as the exploration program evolves. At some point it may be reasonable to relax the requirements, but this should only be done after careful scientific review by an independent body.

#### Recommendation – Sample Evaluation

A research facility for receiving, containing, and processing returned samples should be established as soon as possible once serious planning for a Mars sample return mission has begun. At a minimum, the facility should be operational at least two years prior to launch. The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization. An advisory panel of scientists should be constituted with oversight responsibilities for the facility.

It was evident from the Apollo experience that the science team, and therefore the lunar receiving facility as a whole, would have been more effective if the team members had had prior experience working together as a group on common problems before receiving lunar samples. During the preliminary study of those samples, loss of containment and compromise of quarantine occurred on several occasions. Some of these occurrences might have been avoided had the science team and the receiving facility been operational well before return of the samples.

To avoid similar problems during the initial investigation of returned martian samples and to provide sufficient time to develop and validate the requisite life detection, containment, and sterilization technologies, the receiving facility and its associated science team should be established well in advance of the launch of any sample return mission. The facility should include appropriately stringent biological containment capability and be staffed by a broadly multidisciplinary team of scientists. When fully constituted, the science team should strive to include diverse expertise in such areas as effective biological containment, geological and biological sample processing and curation, microbial paleontology and evolution, field ecology and laboratory culture, cell and molecular biology, organic and light stable isotope geochemistry, petrology, mineralogy, and martian geology.

#### Recommendations – Program Oversight

1. A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample return missions. The panel should be in place at least one year prior to the establishment of the sample-receiving facility (at least three years prior to launch).

"... to coordinate regulatory and other oversight responsibilities, NASA should establish a panel analogous to the Interagency Committee on Back Contamination that coordinated regulatory and oversight activities during the lunar sample return missions. To be effective, planetary protection measures should be integrated into the engineering and design of any sample return mission, and, for an oversight panel to be in a position to coordinate the implementation of planetary protection requirements, it should be established as soon as serious planning for a Mars sample return mission has begun. For the panel to be able to review and approve any plans for a Mars sample-receiving facility, the panel should be in place at least one year before the sample-receiving facility is established."

2. An administrative structure should be established within NASA to verify and certify adherence to planetary protection requirements at each critical stage of a sample return mission, including launch, reentry, and sample distribution.

An internal administrative structure, with clearly defined lines of authority, is required to verify and certify adherence to planetary protection requirements at each critical stage of a sample return mission, including launch, reentry, and sample distribution. The certification should be sequential. That is, the mission should not be allowed to proceed to the next stage until planetary protection requirements for that stage and each preceding stage have been met. For example, reentry should not be authorized unless containment has been verified or the material to be returned has been sterilized. The required internal structure is already partly in place at NASA, but the lines of authority should be more clearly specified and a certification process should be implemented for each mission stage.

3. Recommendation: Throughout any sample return program, the public should be openly informed of plans, activities, results, and associated issues.

In light of the public's past response to other controversies involving science and technology, it is possible that environmental and quality-of-life issues will be raised in the context of a Mars sample return mission. If so, it is likely that the adequacy of NASA's planetary protection measures will be questioned in depth. The most effective strategy for allaying fear and distrust is to inform early and often as the program unfolds. Acknowledging the public's legitimate interest in planetary protection issues, and thereby keeping the public fully informed throughout the decision-making process related to sample return and handling, will go a long way toward addressing the public's concerns.

**Summary of: "Mars Sample Quarantine Protocol Workshop," D.L. DeVincenzi, J. Bagby, M. Race, and J.D. Rummel, Editors, NASA Ames Research Center, Moffett Field, California, April 1997, NASA CP-1999-208772 (1999).**

#### Reason Written

In 1996, several NASA-sponsored studies were underway to look at various aspects of a Mars Sample Return (MSR) mission. One of these studies by the Mars Exploration Long Term Science Working Group (MELTSWG) determined the need for additional study of five specific areas related to Planetary Protection (PP). One of the priority areas identified was the need to develop guidelines for return sample containment and quarantine analysis. In response to this need, the Mars Sample Quarantine Protocol Workshop was convened in June 1997 to deal with three specific aspects of the initial handling of a returned Mars sample: 1) biocontainment, to prevent uncontrolled release of sample material into the terrestrial environment; 2) life detection, to examine the sample for evidence of live organisms; and 3) biohazard testing, to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere.

#### Background

In order to constrain the scope of the Workshop, several starting assumptions were given: 1) The Mars Sample Return mission (MSR) will be launched in the 2005 opportunity; 2) the mission will return samples from biologically interesting sites based on data returned from missions in 1996, '98, '01, and '03; 3) in a nominal mission, the sample will not be sterilized prior to return to Earth; 4) the amount of sample available for quarantine tests will be a small fraction of the total amount returned; and 5) biocontainment of the unsterilized sample will be maintained until quarantine testing for biohazards is accomplished.

### Containment Findings

The Containment Sub-group discussed the development of recommendations that might be adopted by NASA for the safely controlled management of a Mars sample while a quarantine protocol is executed. Containment was defined as: "a system of protection of: 1) the Earth's biosphere from release of 'biological entities' of martian origin, and 2) the integrity of the sample."

### Containment Recommendations

1. **Sample Return Canister:** The entire system of containment – from Mars to Earth – must prevent the escape of potentially hazardous material. This means special design considerations for the canister and planning for Earth return procedures. Specific recommendations include:

- Decontamination of the exterior of the canister that contacts the martian surface;
- Contingencies for non-nominal events (i.e., initial trajectory of Earth return vehicle biased to miss Earth; indicator system to monitor for breach of containment en route; on board system for sterilization in case of an in flight breach in containment; provisions to determine if a breach occurs during a hard impact at the landing site, and suitable sterilization for that event.)

Upon recovery of the canister and reconfirmation of proper containment, the canister must be transported to a quarantine facility in a container meeting regulatory requirements for safe transport of potentially hazardous biological material. Precautions for handling the sample return canister should include provisions for protective garments for the recovery crew and coordination with appropriate regulatory agencies such as USDA-APHIS and EPA.

2. **Mars Receiving Laboratory (MRL):** The unknown nature of any possible hazardous material in the sample warrants the use of the most stringent containment presently afforded to the most hazardous biological entities known on Earth; that is, a Biosafety Level 4 (BSL-4) operation. Appropriate containment is attained through the application of primary and secondary containment principles:

- Primary containment will be provided by utilizing Class III biosafety cabinets – comprised of glove boxes connected in sequence with sealable doors between cabinets and maintained under negative pressure.
- Secondary containment will be provided by the building: a 'high-end' BSL-3 structure which is sealed and maintained under negative pressure, with high efficiency particulate air (HEPA) filtered exhaust air, sterilized waste water, and with provision for personnel showers and appropriate use of disinfectants.
- While biological safety and physical security must be the prime considerations in the design of a Mars receiving facility, there could be alternative approaches to accomplish the needed containment besides a dedicated new facility. One such alternative includes providing a small MRL facility beside an existing approved BSL-4 laboratory (e.g. USAMRIID at Fort Detrick, Maryland, or CDC in Atlanta, Georgia) This would offer flexibility, availability of trained professionals and support staff, and possible simplification of the permit and approval process. Disadvantages include the possible reduction in control of samples by NASA while in the hands of another agency, and background organic residues and contamination that could interfere with sample interpretation. Existing policy for the transport and receipt of potentially hazardous agents requires CDC review of the facility, thereby providing an additional check on safety. Whatever alternative is selected, at least five years must be allowed for the construction and certification of such a highly technical

facility and for the training of professional and support staff. Training periods are required for qualified personnel to become familiar with the new facility so operations are safe, efficient, and accurate.

- Glove box systems can be flexibly designed to include any laboratory equipment required by the protocols. Operational parts of equipment can be housed within the primary containment glove boxes, with electronics, control panels, etc. located outside the primary containment barrier. The report provides preliminary details on the need for careful planning of the sequence of steps for handling and opening the sample canister in the cabinet lines to avoid contamination on contained samples.

### Containment Research and Technology Needs

Specific research areas recommended to accomplish successful containment both in transit and in the laboratory include:

1. Challenge tests of HEPA filtration system should be undertaken using carbon-bearing particles from 10 nm to 100 nm in size.
2. Research should be conducted to choose appropriate isotopes and particle sizes for use in flight verification and testing of canister seals (e.g., carbon compounds, radioactive-tagged particles).
3. Select an appropriate indicator for canister seal integrity upon recovery
4. Design effective processes to clean containment area of terrestrial biological entities and organics to avoid confusion during observations of the Mars samples.
5. Systems must be developed and tested to maintain sample integrity when obtaining aliquots of material for quarantine testing
6. Design research to provide a system for needle puncture of the 'head space' through a vacuum-sealed line; HEPA filters could be incorporated.
7. Determine the suitable sterilization methods for the Mars sample.

### Life Detection Findings

The Life Detection Sub-group was assigned the task to develop a series of tests (a protocol) to detect the presence of live organisms, or of materials that have been derived from live organisms, in samples of material returned from Mars. The group first considered the likely aspects of viable organisms that might be detected and then determined the philosophy that should guide the life detection protocol, which in turn would dictate the sequence, techniques and handling requirements for the protocol. The Sub-group also made recommendations on research needed to refine the eventual protocols.

The philosophy espoused by the Sub-group aimed not only at detecting life, but distinguishing between potential martian life forms and terrestrial contamination. In particular: 1) there must be multiple lines of evidence to support an hypothesis that detected life is of martian origin, and 2) it is essential to understand the geological and potential ecological context of a sample in order to understand the nature of life that might be detected in the samples. A strong quality assurance and quality control program was deemed essential, involving the use of chemical tracers in order to correlate the 'detected' material/organism(s) with the phase of the mission in which material was obtained.

In order to establish the appropriate context for life detection in a sample, a preliminary analysis of the sample was recommended to:

1. Characterize the bulk mineralogy of the sample,
2. Establish its elemental composition,
3. Inventory the volatile and organic materials it may contain,
4. Measure the redox couples present in the sample material, and
5. Obtain a microscopic characterization of the sample surface and interior.

As long as an adequate sterilization method could be defined which would not affect the results of the analysis, the Sub-group felt most of these analyses would not require the sample to be held in biological containment.

### Life Detection Recommendations

The Life Detection Sub-group prioritized three basic methods for accomplishing life detection:

1. Organic chemical analysis and detection including search for functional groups containing reduced carbon, sulfur or nitrogen; analysis of possible kerogen materials for stable isotope abundances; detection of amino acids or possible proteins; analysis for amphiphiles in the form of fatty acids, hopanes, etc; a search for carbohydrates, nucleic acid bases, and related compounds (e.g., DNA, RNA, PNA, etc.); and potential detection of integrated cell walls or cell wall components such as lipopolysaccharides. Assuming current improvements in available technologies, it was felt that cellular life could be detected routinely at the level of 10-100 cells in a sample and as little as one cell in a 100 g sample.
2. Light and/or electron microscopy to detect morphological indications of life, along with the trace mineralogy of the sample. Coupled with staining methods to reveal chemical evidence of life in conjunction with morphological methods, light microscopy was seen as having advantages over electron microscopy in terms of sample preparation, handling and real-time testing. Electron microscopy, particularly ion-probe techniques, can provide critical composition information about samples. The issue of what constitutes a 'representative' sample will need to be defined.
3. Culturing of martian materials and/or living organisms: Although it will be difficult to generalize for putative martian organisms, cultivation as a life detection approach was recommended because of the potential to amplify the presence of life in a sample, to discriminate between a viable organism and materials that were once associated with biology (but not now alive), and to provide a natural link to hazard detection analyses. Attempted cultivation techniques should include not only conditions commensurate with the environment from which samples were obtained, but also the use of multiple media and carbon sources under both aerobic and anaerobic conditions, using both intact samples and processed sample materials. Given the low culturability of environmental microbes from Earth (~1%), culturability is of secondary or tertiary priority for life detection.

### Life Detection Protocol

The Protocol should be an integrated facet of the comprehensive analysis of samples for atmospheric, geophysical, and exobiological purposes. A comprehensive process for sample analysis and life detection was outlined which includes detailed comments about particular steps in the process such as the sample container, sample receiving, sample separation, microscopic/mineralogical/geochemical survey, life detection microscopy, and chemical

analyses for signs of life. The Life Detection Sub-group recommended that the following considerations form the basic concept of chemical analysis techniques in life detection:

1. Seek functional groups important for energy transfer rather than live biomass
2. Seek to identify accumulated biomass-type molecules and cellular components rather than cells or single living entities
3. Use more sensitive and less selective detectors for the first sample screening procedure. Rather than employing the selectivity of GC-MS or KC-MS as the first step, use highly sensitive infrared micro-calorimetric or lab-on-a-chip technology to provide high sensitivity detection of functional groups.
4. Integrate remnant parts as a preliminary indication of possible extant life (the amount of functional groups remaining from remnant parts often exceeds the live biomass in samples on Earth.)
5. It may not be possible to rely on DNR, RNA, proteins or even carbon-based molecular backbones as indicators because extraterrestrial life may be markedly different in detail from life on Earth. Focus initial screening efforts on amine and carboxyl functional groups to detect signs of life based on any backbone, C, N, P, S or Si. Comparison of stable isotopic signatures of non-life-like compounds (e.g., polycyclic aromatic hydrocarbons, PAHs) and life-like compounds may provide additional information on the potential existence of life on Mars.

#### Life Detection Research and Technology Needs

NASA must begin to incorporate life detection technologies into planning and anticipated sample receiving activities for MSR. In particular, a plan must be developed for the acquisition and operation of appropriate instrumentation within the sample handling facility, and appropriate sterilization protocols and methods must be developed to prepare samples for distribution to the wider scientific community.

#### Biohazard Testing Findings

The Biohazard Testing Sub-group was assigned the task of developing an up-to-date methodology to determine if returned martian sample materials are hazardous, regardless of whether life or biological entities are detected. The Sub-group proposed a tiered or stepwise approach to testing based heavily on protocols used by research and agencies for a wide range of biological agents. These tests would: 1) focus on a broad range of biohazards, 2) screen for indication of biological activity or disruption thereof, and 3) incorporate systematic feedback as data are gathered from the life detection studies, chemical analyses, and biohazard tests themselves. Emphasis was placed on hazards posed by organisms that replicate because of their potential for large scale negative impacts on Earth's ecosystems.

Two priority biohazard concerns were addressed: pathogenicity and ecological disruption. (Chemical toxicity was not considered a significant biohazard or global threat since toxic materials will not replicate and spread, and since proper laboratory protocols will protect those who work with the samples). Detailed information and discussion about various tests are provided in the appendix of the report. In general, the Sub-group recommended the following:

#### Biohazard Testing Recommendations

**Pathogenicity:** Regardless of the outcome of preliminary life detection tests or chemical analyses, it will be prudent to screen samples for two types of pathogenicity – toxic and

infectious – using tests specifically designed to detect biological activity or disruptions. *In vitro* methods are considered superior to whole organism tests for preliminary biohazard screening because of their sensitivity, simplicity and speed, as well as their widespread use, acceptance and interpretation. By selecting a suitably diverse range of *in vitro* tests and conditions, it will be possible to screen for biologically important outcomes that might be indicative of biohazards in a wide range of representative species and taxonomic groups. It would be advisable to include a range of *in vitro* tests that are routinely used by agencies and researchers when scanning for pathogenesis. In addition, the inclusion of two additional types of tests – a series of laboratory mice injection studies (because of their extensive use for pathogenicity and biohazard testing) and a series of tests using *Tetrahymena* (as a model for metazoan biochemistry) – were discussed. A recommended battery of tests for detection indication of potential pathogenicity in the sample might include:

1. Diverse microbial media that use varied laboratory initial conditions
2. Selected tissue cultures and cell lines from mammalian organ systems, fish and insects
3. Embryonating chicken eggs
4. Mouse injection studies
5. *Tetrahymena* (protozoans)
6. Plant tissue cultures (wheat, rice, potato).

**Ecological Disruption:** In the event of inadvertent introduction to the Earth's biosphere of putative martian microbes, there would be little threat of widespread ecological disruption based on our comparative knowledge of martian and Earth conditions and our knowledge about microbial potential on Earth. Nevertheless, since the risk of potentially harmful effects is not zero, it will be prudent to screen for the ability of the returned sample to disrupt microbial ecosystems. Although such tests are not routinely done, it would be advisable to design and conduct suitable microcosm tests to screen for potential ecosystem effects or disruption in biogeochemical cycles. Two types of microcosm tests are recommended, the first designed to assay for disruptions of important representative microbial systems upon addition of martian material, and the second to determine if any undetected biological entities can grow or propagate in selected sterilized microcosm of representative terrestrial ecosystems

**Criteria for Distribution of Martian Samples:** The Biohazard Testing Sub-group considered the many possible interpretations of data for the proposed battery of life detection and biohazard tests and developed a table providing an overview of various combinations of findings (Table 1 in report). In general, if any life forms are detected, even if preliminary test suggest they do not pose a biohazard, the Sub-group advised continued strict containment, rather than controlled distribution, at least initially. Strict containment should be maintained in light of any positive test results until findings are verified and/or a scientific panel provides further guidance on subsequent handling. All verification testing should use only *in vitro* tests under BSL-4 containment. No consensus was reached on what containment/release recommendations should be made if all life detection and biohazard tests are negative. Additional discussion will be needed to translate the various test outcomes into specific recommendations for release of unsterilized materials from containment.



Biohazard Research and Technology Needs: Specific recommendations for research and development related to biohazard testing were identified in the following areas:

1. Validation of methodological approach (cell and tissue test rather than whole organisms studies; pre-testing of efficacy; techniques for characterizing any isolated or suspected life forms etc.)
2. Microcosm Research (development, effectiveness; predictive value; non-destructive, long-term observation and sampling, etc.)
3. Representative samples, controls and replicates
4. Other operational issues (training and monitoring programs for lab personnel; management of lab operations and facilities; issues related to limited quantities of material, sample allocation, research access, and evaluation of research proposals).

**Summary of: "Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies," Task Group on Sample Return from Small Solar System Bodies, chaired by Leslie Orgel, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1998).<sup>12</sup>**

#### Reason Written

With the advent of possible sample return missions from multiple planetary bodies, NASA asked the Space Studies Board (SSB) of the National Research Council (NRC) in 1997 to assess the potential for a living entity to be contained in or on samples returned from planetary satellites and other small solar system bodies such as asteroids and comets. The Task Group on Sample Return from Small Solar System Bodies was asked to build on and extend earlier SSB studies on Mars (1992 forward-contamination report [SSB 1992] and 1997 sample return report [SSB 1997]) and address the following specific tasks:

1. Assess the potential for a living entity to be contained in or on samples returned from planetary satellites or primitive solar system bodies, such as asteroids, comets, and meteoroids;
2. Identify detectable differences among small solar system bodies that would affect the above assessment;
3. Identify scientific investigations that need to be conducted to reduce the uncertainty in the above assessment; and
4. Assess the potential risk posed by samples returned directly to Earth from spaceflight missions, as compared to the natural influx of material that enters Earth's atmosphere as interplanetary dust particles, meteorites, and other small impactors.

#### Background and Study Approach

Because there is no direct evidence that a living entity evolved or exists on any small solar system body, the task group examined indirect evidence based on data from Earth, meteorites, and the Moon and on astronomical observations of distant objects in an effort to assess whether NASA needs to treat samples returned from small solar system bodies differently from samples returned from Mars. To identify the requirements for the origin and survival of living organisms, the task group examined contemporary views on the range of conditions under which life can originate, the conditions required for the preservation of metabolically active organisms in terrestrial environments, and the somewhat different conditions needed to preserve living

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12. Available on line: [www.nas.edu/ssb/ssb.html](http://www.nas.edu/ssb/ssb.html) (click on 'Reports' and '1998').

organisms in a dormant form. Based on this analysis, the task group identified six parameters (liquid water, energy sources, organic compounds, temperature, radiation intensity, and natural influx to Earth) as relevant to its assessment and formulated the following six questions to help determine how returned samples should be handled:

1. Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?
2. Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?
3. Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO<sub>2</sub> or carbonates and an appropriate source of reducing equivalents) in or on the target body to support life?
4. Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., >160°C)?
5. Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life forms?
6. Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

In applying the questions, the task group drew on existing data on the origin, composition, and environmental conditions (past and present) of each small body or planetary satellite examined and then determined whether the quality and weight of the evidence were convincing enough to allow making judgments and deriving findings. The answers to the questions, taken together, were used to reach a considered conclusion that the potential for a living entity to be in or on a returned sample was either 'negligible' or 'not negligible.' Because of the incomplete current state of knowledge about small solar system bodies, there are no definitive answers to the questions, and so all judgments regarding biological potential are qualitative (not quantitative).

The questions allow for a conservative, case-by-case approach to assessing whether or not special physical and biological isolation and handling of returned samples (containment) would be warranted, taking into account information about the different small bodies, natural influx to Earth of material from small bodies, and the possible nature of putative extraterrestrial life. An answer of 'yes' to any question argues against the need for special containment beyond what is needed for scientific purposes. For containment procedures to be necessary, an answer of 'no' needs to be returned to all six questions. For such samples, strict containment and handling would be required, similar to the Mars sample return handling recommended by the SSB in its 1997 report [SSB 1997].

The task group chose to consider only two possible alternatives for containment and handling of samples returned from small solar system bodies, either: 1) strict containment and handling of returned samples as outlined in the Mars report [SSB 1997], or 2) no special containment beyond what is needed for scientific purposes. The task group ruled out intermediate or compromise procedures involving partial containment.

## Findings

**Planetary Satellites:** Satellites are natural consequences of planetary formation processes. The task group considered the possibility of sample return from the major satellites of the innermost planets including the satellite of Earth (the Moon), satellites of Mars (Phobos and Deimos), and selected satellites of Jupiter (Io, Europa, Ganymede, and Callisto). The potential for a living entity to be present in samples returned from the Moon and Io is negligible. The potential for a living entity to be present in samples returned from Phobos, Deimos, and Callisto is extremely low, but the task group could not conclude that it is necessarily zero. Importantly, the task group found that there is a significant potential for a living entity to be present in samples returned from Europa and Ganymede.

**Asteroids:** Asteroids are the remnants of planetesimals – small primordial bodies from which the planets accumulated. Common asteroid types include undifferentiated, primitive types (C-, B-, and G-types); undifferentiated metamorphosed types (Q- and S-types [ordinary chondrites]); and differentiated types (M-, V-, J-, A-, S- [stony irons], and E-types). Other types of asteroids have been defined, including the common P- and D-types in the outer parts of the asteroid belt, but little is known about their composition and origin. Others are subdivisions of the types listed above, whereas still others are rare, new types, generally seen only among the population of very small asteroids. For undifferentiated, primitive (C-type) asteroids, the potential for a living entity to be contained in returned samples is extremely low, but the task group could not conclude that it is necessarily zero. Because of a fundamental lack of information about P- and D-type asteroids, the potential for a living entity to be present in returned samples cannot be determined and, therefore, was considered conservatively by the task group as possible at this time. For all C-type asteroids, undifferentiated metamorphosed asteroids, and differentiated asteroids, the potential for a living entity to be present in returned samples is extremely low, but the task group could not conclude that it is necessarily zero.

**Comets:** Comets are believed to have formed in the protoplanetary disk, at distances from the Sun ranging from the distance of proto-Jupiter to far beyond the distance of proto-Neptune. It is unlikely that a living entity could exist on comets, but the possibility cannot be completely ruled out except in a few cases, such as in the outer layers of Oort Cloud comets entering the solar system for the first time. Thus, the potential for a living entity to be present in returned samples from all comets was considered by the task group to be extremely low, but the task group could not conclude that it is necessarily zero.

**Cosmic Dust:** Because interplanetary dust particles (IDPs) are derived from a variety of sources, including interstellar grains and debris from comets, asteroids, and possibly planetary satellites, IDPs cannot be viewed as a distinct target body. As a result, the assessment approach used in this study does not lend itself readily to IDPs. Instead, the task group considered the potential source(s) of any IDPs that might be returned in samples. For the purposes of this study, IDPs are viewed as originating from either a single identifiable parent body or multiple sources. Particles collected near a particular solar system body are viewed as originating from that body, possibly including grains recently released from that body. Thus, the potential for a living entity to be present in returned samples, and the associated containment requirements, will be the same as those for the parent body. On the other hand, IDPs collected in the interplanetary medium may represent a mixture of dust originating from many parent bodies. Because IDPs in the

interstellar medium are exposed to sterilizing doses of radiation, the potential for IDPs to contain viable organisms or a living entity is negligible.

### Conclusions and Recommendations

Table ES.1 summarizes the task group's assessment of the level of containment and handling warranted for samples returned from the planetary satellites and small solar system bodies examined in this study. The table summarizes the requirements that apply to samples for which strict containment and handling are advisable. It is important to note that the task group's recommended approach is provided only as a guide and not as an inflexible protocol for determining whether containment is required. The final decision must be based on the best judgment of the decision makers at the time and, when possible, on experience with samples returned previously from the target bodies.

### Recommendations – Containment of Returned Samples

1. On the basis of available information about the Moon, Io, dynamically new comets (specifically the outer 10 meters), and interplanetary dust particles (sampled from the interplanetary medium, sampled near the Moon or Io, or sampled in a way that would result in exposure to extreme temperatures), the task group concluded with a high degree of confidence that no special containment is warranted for samples returned from those bodies beyond what is needed for scientific purposes.

For samples returned from Phobos and Deimos, Callisto, C-type asteroids, undifferentiated metamorphosed asteroids, differentiated asteroids, and comets other than dynamically new comets, the potential for a living entity in or on a returned sample is extremely low, but the task group could not conclude that it is zero. Based on the best available data at the time of this study, the task group concluded that containment is not warranted for samples returned from these bodies or from interplanetary dust particles collected near these bodies. However, this conclusion is less firm than the conclusion for the Moon and Io and should be reexamined at the time of mission planning on a case-by-case basis.

2. For samples returned from Phobos and Deimos, Callisto, C-type asteroids, undifferentiated metamorphosed asteroids, differentiated asteroids, comets other than dynamically new ones, and interplanetary dust particles sampled near these bodies, a conservative, case-by-case approach should be used to assess the containment and handling requirements. NASA should consult with or establish an advisory committee with expertise in the planetary and biological sciences relevant to such an assessment. The goal of such an assessment should be to use any new, relevant data to evaluate whether containment is still not warranted. This assessment should take into account all available information about the target body, the natural influx to Earth of relevant materials, and the likely nature of any putative living entities. Such an advisory committee should include both NASA and non-NASA experts and should be established as early in the mission planning process as possible.

For samples returned from Europa and Ganymede, the task group concluded that strict containment and handling requirements are warranted. Because the knowledge base for P- and D-type asteroids is highly speculative, the task group concluded conservatively that strict containment and handling requirements are warranted at this time. Strict containment and handling requirements are also warranted for interplanetary dust particles collected near these bodies unless they are sampled in a way that would result in exposure to extreme temperatures, e.g., spike heated.

TABLE ES.1: Summary of Currently Recommended Approach to Handling Samples Returned from Planetary Satellites and Small Solar System Bodies Assessed by the Task Group on Sample Return from Small Solar System Bodies

I. No Special Containment and Handling Warranted Beyond What Is Needed for Scientific Purposes		II. Strict Containment and Handling Warranted
Ia. High Degree of Confidence	Ib. Lesser Degree of Confidence <sup>a</sup>	
The Moon Io Dynamically new comets <sup>b</sup> Interplanetary dust particles <sup>c</sup>	Phobos Deimos Callisto C-type asteroids Undifferentiated metamorphosed asteroids Differentiated asteroids All other comets Interplanetary dust particles <sup>e</sup>	Europa Ganymede P-type asteroids D-type asteroids Interplanetary dust particles <sup>d</sup>

- a. Sub-column Ib lists those bodies for which confidence in the recommended approach is still high but for which there is insufficient information at present to express it absolutely. This lesser degree of confidence does not mean that containment is warranted for those bodies; rather, it means that continued scrutiny of the issue is warranted for the listed bodies as new data become available. The validity of the task group's conclusion that containment is not warranted for the bodies listed in Ib should be evaluated, on a case-by-case basis, by an appropriately constituted advisory committee in light of the data available at the time that a sample return mission to the body is planned.
- b. Samples from the outer 10 meters of dynamically new comets.
- c. Interplanetary dust particles sampled from the interplanetary medium and from the parent bodies listed in sub-column Ia.
- d. Interplanetary dust sampled from the parent bodies in column II and collected in a way that would not result in exposure to extreme temperatures.
- e. Interplanetary dust sampled from the parent bodies listed in sub-column Ib.

3. Based on currently available information, samples returned from Europa, Ganymede, P- and D-type asteroids, and interplanetary dust particles sampled near these bodies should be contained and handled similarly to samples returned from Mars [SSB 1997]. Interplanetary dust particles sampled in a way that would result in exposure to extreme temperatures, e.g., spike heated, should not be contained or handled in a special way beyond what is needed for scientific purposes.

#### Handling of Returned Samples

For samples that are returned from planetary satellites and small solar system bodies and that warrant containment, the concerns about biohazards or large-scale adverse effects on Earth are similar to those identified earlier for Mars [SSB 1992]. The task group concluded that the risks of pathogenicity from putative life forms are extremely low, because it is highly unlikely that extraterrestrial organisms could have evolved pathogenic traits in the absence of host organisms. However, because there are examples of opportunistic pathogens from terrestrial and aquatic environments that have not co-evolved with their hosts, the risk cannot be

described as zero. The recommendations on containment and handling in the Mars report [SSB 1997] represent a strong basic framework for addressing potential risks associated with returned samples warranting containment.

The microbial species composition of most anaerobic environments on Earth is not known, and consequently it is also not known how the species composition of these anaerobic microbial communities might change over time, what environmental factors might influence these changes, or what the incidence of and successful colonization by new species of microorganisms in these habitats might be. Accordingly, the task group concluded that although there is a low likelihood of a viable anaerobic microorganism surviving transport through space and finding a suitable anaerobic habitat on Earth, growth in a suitable habitat if found might be possible. This conclusion is necessary because of the current lack of information about anaerobic environments on Earth that may be analogous to environments on other solar bodies, and the likelihood that the metabolic properties of such an extraterrestrial anaerobe would resemble an Earth anaerobe from a similar environment.

For overall evaluation of returned samples that warrant containment, it will be necessary to apply a comprehensive battery of tests combining both life-detection studies and biohazard screening.

#### Recommendations for Sample Handling

1. Returned samples judged to warrant containment should be quarantined and screened thoroughly for indications of a potential for pathogenicity and ecological disruption, even though the likelihood of adverse biological effects from returned extraterrestrial samples is very low.
2. NASA should consult with or establish an advisory committee of experts from the scientific community when developing protocols and methods to examine returned samples for indicators of past or present extraterrestrial life forms.
3. The planetary protection measures adopted for the first sample return mission to a small body whose samples warrant special handling and containment should not be relaxed for subsequent missions without a thorough scientific review and concurrence by an appropriate independent body.

#### Scientific Investigations to Reduce Uncertainty

The task group identified various issues for which scientific research could help to reduce the uncertainty in its assessment of the potential for a living entity to be contained in or on samples returned from planetary satellites and small solar system bodies. (these general suggestions are incorporated into the text of Chapters 2-6) However, one topic is of sufficient importance that it requires emphasis.

Because organisms subjected to sterilizing conditions for a sufficient time period pose no threat to terrestrial ecosystems, it is important to assemble a database on the survival capacity of a wide range of terrestrial organisms under extreme conditions. Despite the existence of a rich literature on the survival of microorganisms exposed to radiation and high temperatures, the studied taxa represent only a small sampling of the microbial diversity known to exist in the biosphere and, in general, have not been taken from extreme environments. Little is known about the radiation and temperature resistance of microorganisms from environments on Earth

that have the chemical and physical characteristics likely to be encountered in or on small solar system bodies.

#### Recommendations Concerning Investigations to Reduce Uncertainty

NASA should sponsor research that will lead to a better understanding of the radiation and temperature resistance of microorganisms from environments on Earth that have the chemical and physical characteristics likely to be encountered in or on small solar system bodies. Information on the survival of organisms subjected to long- or short-term ionizing radiation needs to be collected for both metabolically active and dormant stages of diverse groups of microorganisms, including hyperthermophiles, oligotrophic chemoorganotrophs, and chemolithoautotrophs. Likewise, it is important to establish short - and long-term temperature survival curves for similarly broad groups of metabolically active and dormant organisms. In particular, data are required on survival of diverse microorganisms under flash heating (1- to 10-second exposures) to temperatures between 160°C and 400°C.

**Summary of: "Mars Sample Handling and Requirements Panel (MSHARP) Final Report," Michael H. Carr, et al., NASA Jet Propulsion Lab, Pasadena, CA, NASA TM-1999-209145 (1999).**

#### Charter

In anticipation of the return of samples from Mars, NASA's Office of Space Sciences chartered a panel to examine how Mars samples should be handled. The panel was to make recommendations in three areas: 1) sample collection and transport back to Earth; 2) certification of the samples as non-hazardous; and 3) sample receiving, curation, and distribution. This report summarizes the findings of that panel.

#### Background

The samples should be treated as hazardous until proven otherwise. They are to be sealed within a canister on Mars, and the canister is not to be opened until within a Biosafety Hazard Level 4 (BSL-4) containment facility here on Earth. This facility must also meet or exceed the cleanliness requirements of the Johnson Space Center (JSC) facility for curation of extraterrestrial materials. A containment facility meeting both these requirements does not yet exist. Hazard assessment and life detection experiments are to be done at the containment facility, while geochemical characterization is being performed on a sterilized subset of the samples released to the science community. When and if the samples are proven harmless, they are to be transferred to a curation facility, such as that at JSC.

#### Summary and Conclusions

1. The search for evidence of life, particularly past life, is a primary objective of the Mars exploration program. Parallel and intimately connected goals are determination of the planet's climate and of the planet's geologic histories.
2. Many of the outstanding biologic, climatologic, and geologic issues with respect to Mars are unlikely to be resolved until we have a variety of resumed samples.
3. The present martian surface is very hostile to life because of its low temperatures, the lack of liquid water, the high UV flux, the presence of oxidants, and the scarcity of organics.

4. The chances of finding extant life in samples returned from the martian surface are very low, and even if extant life were present, it would be unlikely to have significant ecological impact or other harmful effects on the Earth. The risk is not zero, however.
5. Because we cannot demonstrate that the risk is zero, the returned samples should be assumed to be potentially harmful until proven otherwise. They should be placed in sealed containers on Mars, and the containers should be opened only in a BSL-4 containment facility here on Earth. No samples should leave BSL-4 containment unless sterilized or proven to be harmless.
6. Return of samples to the International Space Station is impractical and is likely to be more risky than returning them to Earth.
7. Sterilizing samples at Mars is not advocated because sterilization would be difficult to accomplish and verify remotely on Mars, and sterilization would destroy much of the biologic and climatologic information in the samples.
8. We endorse the current Athena sample acquisition plan to use a rover to acquire primarily rock cores, with a few additional soil samples. We strongly advocate acquisition of a contingency sample by the lander, although this need not be returned if the rover mission is successful.
9. The sampling strategy should be aimed at acquiring the maximum variety of samples from the sites visited.
10. Contamination of the samples with terrestrial materials is of considerable concern because it could compromise the science results from the samples. Also, any false positives on hazard assessment and life detection tests would confuse interpretation of analytical results from the samples and could significantly delay release of unsterilized samples from BSL-4 containment for distribution to the science community.
11. All components that land on the martian surface must be cleaned to at least Pathfinder levels of cleanliness.
12. All spacecraft components that touch the samples must be sterilized and cleaned to significantly higher standards than Pathfinder.
13. Recognizing that some contamination of the samples could occur, we strongly advocate the use of tracers, witness plates, and assays to help identify adventitious contaminants. We do not, however, advocate deliberately impregnating the drill bits with tracers because of concerns that contamination of the samples by the tracers would be significant and would interfere with sample analysis.
14. The sample canister must be sealed before leaving the martian surface, and the integrity of the seal should be confirmed either before leaving the martian surface or while in orbit at Mars.
15. The sample canister must be transferred to the Earth Return Vehicle (ERV) in such a way that the only martian materials on the ERV are those sealed within the sample canister.
16. Insofar as it is practical during return to Earth, the samples should be maintained at temperatures no higher than 240 K, the maximum temperature they are likely to have experienced on Mars. It is especially desirable that the samples not be allowed to experience temperatures above 270 K.
17. We recommend that introduction of unsterilized material into the Earth's environment be kept to a very low probability, mainly by system design, such as by multiple seals and interleaved filters, rather than through monitoring containment and incorporating various contingency responses into the design. We believe the most likely times of containment failure are at the surface of Mars, when a decision could be made not to return the samples, and during entry and landing at Earth, when monitoring has little value. Limited resources are better used by designing against failure rather than by monitoring and contingency mechanisms.



18. After reaching Earth, the sample canister must be opened in a sample receiving facility (SRF) with the equivalent of BSL-4 containment. The facility must also meet the cleanliness standard used for handling extraterrestrial materials at JSC. To our knowledge, no such facility now exists.
19. We view the SRF as primarily a service facility for the science community, rather than a research facility. The facility will make an early inventory of the samples, do some preliminary hazard assessment and life detection testing, and sterilize a subset of the samples for distribution to the science community for geochemical characterization.
20. Early distribution of a subset of sterilized samples is an essential element in both scientific analysis of the samples and in assessing their potential for harm. The geologic and geochemical characteristics of the samples, such as the presence and nature of any organics, will be important for deciding what hazard and life detection testing needs to be done. Geochemical characterization is most reliably and comprehensively done by the at-large science community. Radiation sterilization is the method of choice because of its minimal effects on the geochemical character of the samples. Allocation of the distributed samples should be by the normal NASA Research Announcement (NRA) Peer Review process.
21. Some hazard assessment and life-detection experiments must be done in the SRF. We think it premature to advise how these might best be done, given that technologies will likely evolve considerably between now and 2008 when the first samples return, but we suspect that hazard assessment will primarily involve tissue-cell culture testing rather than tests on whole organisms.
22. Some of the hazard assessment and life-detection experiments could be done at containment facilities other than the SRF by distributing unsterilized samples to other containment facilities using well established procedures for handling and transporting biohazardous materials.
23. The SRF can be scaled, built, and configured in a variety of ways, depending on such factors as what testing is to be done in the facility, as opposed to testing elsewhere, whether the facility is for Mars samples only or for extraterrestrial materials in general, and how long the Mars sample return program is to last. We believe that an SRF built from modular, modest-sized, commercially available, biosafety laboratories is appropriate for the early sample returns. Should life be detected and/or the samples prove to be hazardous, then more elaborate alternatives could be built.
24. The SRF should be built, staffed, and operational 1-2 years before receipt of the samples.
25. If and when the samples are found to be non-hazardous, the samples should be transferred to a curation facility such as that at Johnson Space Center (JSC).

**Summary of: "Size Limits of Very Small Microorganisms: Proceedings of a Workshop," Steering Group for the Workshop on Size Limits of Very Small Microorganisms, A. Knoll and M.J. Osborn, Co-Chairpersons, Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1999).<sup>13</sup>**

### Background

Following the report of possible microfossils ranging in length from 10 to 200 nm in the martian meteorite ALH84001, NASA's Office of Space Science requested that the National Research Council's Space Studies Board organize a workshop to provide a forum for discussions of the theoretical minimum size for microorganisms. The Board formed the Steering Group for the Workshop on Size Limits of Very Small Microorganisms, which convened a workshop on October 22-23, 1998 of leading experts in fields relevant to this question.

The workshop was organized into four panels each addressing a set of distinct but related questions relevant to the size limits of very small organisms. Eighteen invited panelists, representing fields ranging from cell biology and molecular genetics to paleontology and mineralogy, joined with other participants in a wide-ranging exploration of minimal cell size and the challenge of interpreting micro- and nano-scale features of sedimentary rocks found on Earth or elsewhere in the solar system. This NRC report contains the proceedings of the Workshop on the Size Limits of Very Small Microorganisms. It includes position papers presented by the individual panelists, arranged by panel, along with a summary, for each of the four sessions, of extensive roundtable discussions that involved all workshop participants.

### Findings

Panel 1 addressed the following questions:

1. What features of biology characterize microorganisms at or near the nanometer scale?
2. Is there a theoretical size limit below which free-living organisms cannot be viable?
3. If we relax the requirement that cells have the biochemical complexity of modern cells, can we model primordial cells well enough to estimate their likely sizes?

Consensus was reached by Panel 1 participants on the following major points, assuming free-living cells with conventional biochemistry:

1. A minimum of about 250 to 450 essential genes are required for viability.
2. The minimal viable cell diameter is expected to lie in the range of 250 to 300 nm.
3. The number of ribosomes required for adequate genome expression is a significant constraint on minimal cell size.
4. If the requirement for conventional biochemistry and genetics is relaxed, especially with reference to primordial or exobiotic self-replicating systems, the possibility of much smaller cells must be considered.

Panel 2 addressed the following questions:

1. Is there a relationship between minimum cell size and environment?
2. Is there a continuum of size and complexity that links conventional bacteria to viruses?
3. What is the phylogenetic distribution of very small bacteria?

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13. Available on line: [www.nationalacademies.org/ssb/bib1.html](http://www.nationalacademies.org/ssb/bib1.html)

Consistent with the theoretical limits articulated by Panel 1, members of Panel 2 reported that:

1. Bacteria with a diameter of 300 to 500 nm are common in oligotrophic environments, but that smaller cells are not.
2. Nanobacteria reported from human and cow blood fall near the lower size limit suggested by cell biologists; however, the much smaller (ca. 50 nm) bodies found in association with these cells may not, themselves, be viable organisms.
3. Observations on archaea indicate that, in general, they have size limits similar to those for bacteria.

Two problems constrain discussions on minimal cell size in natural environments. Commonly used methods of measuring cell size have inherent uncertainties or possibilities of error. Perhaps more important, most cells found in nature cannot be cultivated. Thus, ignorance about biological diversity at small sizes remains large. These problems notwithstanding, it appears that very small size in modern microorganisms is an adaptation for specific environmental circumstance, including stress and scarcity of resources. Primordial organisms may or may not have been tiny, but the smallest organisms known today reside on relatively late branches of the RNA phylogeny.

Panel 3 addressed the following question:

1. Can we understand the processes of fossilization and non-biological processes sufficiently well to differentiate fossils from artifacts in an extraterrestrial rock sample?

Panel 3 reached a general consensus on the following points:

1. Terrestrial rocks contain an observable and interpretable record of biological evolution, but as we go further back into time, that record becomes attenuated and difficult to interpret in detail. Martian samples may actually be better preserved than terrestrial sediments of comparable age, but lack both modern martian organisms for comparison and a more or less continuous fossil record that connects the present with early planetary history.
2. A better understanding of biological signatures in sedimentary rocks is needed, and it is needed before intelligently collected martian samples are returned to Earth. These signatures certainly include fossil morphologies, but they must also include biomarker molecules, isotopic fractionation, and biological mineralization and trace element concentrations. In all cases, improved understanding of biological pattern formation must proceed in tandem with better knowledge of the generative capacity of physical processes.
3. There is both a need and an opportunity to more effectively integrate laboratory and field observations of fossilization processes with investigations of Earth's early sedimentary record. Multidisciplinary investigations are required in exopaleontological research, and there is a need for new technologies that will enhance our ability to obtain chemical information from individual microstructures.

Panel 4 addressed the following questions:

1. Does our current understanding of the processes that led from chemical to biological evolution place constraints on the size of early organisms?
2. If size is not constrained, are there chemical signatures that might record the transition to living systems?

Panel 4 reported that as yet, there is no consensus view of how life originated. There is, however, broad agreement that the first living systems were far simpler than the simplest free-living organisms known today. The concept that life passed through a stage in which RNA, or a polymer much like it, provided both genetic information and catalysis suggests what such a simple organism might have been like. Organisms characterized by such single-biopolymer chemistry could have been minute, perhaps as small as 50 nm in diameter. This means that the minimum size observable in living cells may not be applicable in setting limits for biological detection on Mars and Europa. The earliest organisms on Earth (or elsewhere) would probably be extremely difficult to recognize as fossils.

### Conclusions

Sometime in the next 10-12 years a small sample of martian rock and soil will be returned to Earth. Among the important questions that will be asked of these samples is: Has Mars ever been a biological planet? Our ability to address this question is directly related to our understanding of the range of morphological features that can be produced by life and by physical processes, as well as the ranges of organic chemicals, mineral forms, and sedimentary rock features that can be generated by biological and by non-biological processes. The results of the workshop make clear a consensus regarding the size and chemical limits of life on Earth.

But, given reasonable uncertainty about whether such features are particular products of terrestrial evolution or universal features of life, the meter stick by which the biogenicity of martian or other planetary samples is measured will likely be knowledge of the limits on physical processes – knowledge that needs to be developed before samples from Mars arrive in the laboratory.

**Summary of: "Current State of Controversy About Traces of Ancient Martian Life in Meteorite ALH84001," Allan H. Treiman, Lunar and Planetary Institute, Houston, Texas, Feb. 2000.**

McKay et al. Hypothesis – Four arguments together suggest that formation of carbonate globules in ALH84001 was associated with martian life [1].<sup>14</sup> In the globules:

1. Polycyclic Aromatic Hydrocarbons, (PAHs, organic material) are martian and characteristic of degraded organic matter.<sup>mg2 15</sup>
2. Mineral assemblages and chemical zoning patterns are characteristic of biologic influence.<sup>e1,s1,s2,s3</sup>
3. Sub-micron magnetite grains have properties indistinguishable from, and unique to, those formed by some Earth bacteria.<sup>t1,t2,t3,t4</sup>
4. Surfaces are decorated with bacteria-shaped objects, inferred to be mineralized remains of bacteria.<sup>s1,s1a</sup>

14. Bracketed numbers in Treiman's summary indicate references, and are keyed to his reference list shown on page 84.

15. Superscripts in Treiman's summary refer to analytical technique(s) and/or instrumentation used, and are keyed to the list on page 82.

Precondition: Carbonate globules formed at temperatures consistent with life. Unproven, but probably true [2,3].<sup>e1,mg1,t1</sup>

#### 1. Organics/PAHs are martian and biogenic?

- Martian? Probably.
  - > Martian origin suggested by intimate mixing with carbonate, decrease in abundance near fusion. [1,4,5]<sup>mg2,x2,x3</sup>
  - > But some contradictory evidence, issue unresolved [4,6-9]<sup>mg1,mg2,mg3,mg4,i2</sup>
  - > Nearly all organic carbon in ALH84001 is terrestrial [9-11].<sup>mg1,mg5,mg6,c1</sup>
- Biogenic? Unproven/'unprovable'?
  - > Similarity to biogenic PAHs inadequately documented.
  - > Similar to PAHs in CM chondrites and IDPs. [12]
  - > Earth weathering/oxidation reduces all PAHs, of any origin, to core molecules [13].<sup>mg7</sup>

#### 2. Mineral Assemblages

- Not diagnostic of biology [3, 14-16]

#### 3. Nanophase magnetites

- BIOGENIC !? Maybe.
  - > Carbonate globules all include two layers with abundant submicron grains of magnetite in a porous (?) matrix of magnesite carbonate.<sup>e1,s1,s1a,t1,t2,t3,t4</sup>
  - > ~1/4 of the magnetites are identical to magnetites from magnetosomes of some magnetotactic bacteria: size, shape, form, structural perfection, lack of chemical substituents [17-19].<sup>t1,t2,t3,t4</sup>
  - > These properties suffice for recognition of magnetites as biogenic, from bacterial magnetosomes [20,21].
- BUT...
  - > Does not explain other 3/4 of submicron magnetite grains.
  - > Does not explain why magnetotactic magnetites are there.
    - Why would magnetotactic bacteria live in rock?
    - If magnetites were transported into rock, how could magnetite-rich layers in globules be so sharp and be so similar through the rock?
    - Abiotic experiments reported to produce magnetites with these "biogenic" properties [16].<sup>e1,x1,s1b,s2,t1,t3?,t4,c2</sup>

#### 4. Bacteria-Shaped Objects

- Visually appealing, scientifically weak
- Some are inorganic
  - > Whisker-shaped magnetites, epitaxially aligned magnetites [22,23]<sup>s1,s1a,t1,t2,t3</sup>
  - > Lamellar protrusions on mineral surfaces [24,25]<sup>s1,s1a</sup>
- Some may be terrestrial
  - > Artifacts of sample preparation? [24]<sup>s1,s1a</sup>

- > Terrestrial objects unknown origin? [26] <sup>s1</sup>
- > Earth organisms? [27,28] <sup>s1a,s2,sf1,i1,b1-b5</sup>
- Some are too small
  - > Objects of diameter <100 nm suspect as bacteria.
  - > Objects suggested to be bacterial appendages or desiccated bacteria [29].
- Limited data.
  - > Few images.
  - > No internal structure.
  - > No chemical compositions.
  - > No sense of community structure.
  - > No sense of ecology.

#### Summary:

1. No argument has been fully validated.
2. Arguments A, B, D weaker than in 1996.
3. Argument C (nanophase magnetites) stronger, but still problematic. A plausible abiotic hypothesis is available.
4. Having all four arguments be true together seems less likely than any single one be true.
5. The nature of scientific evidence.
  - Lack of proof is not disproof.
  - Lack of disproof is not proof.

#### Analytical Method(s)/Instrumentation Applied to Study of ALH84001 (not all in papers cited herein):

- S. Scanning Electron Microscopy
  - s1. Secondary electron imagery – SEM (SEI)
    - s1a. SEI with a field emission electron gun – FEG-SEM
    - s1b. Environmental SEM
  - s2. Back-scattered electron imagery – BSE
  - s3. Chemical analysis by energy dispersive X-ray spectrometry – EDX
- E. Electron Microprobe
  - e1. Electron microprobe chemical analyses, X-ray dispersive spectrometry
  - e2. Element abundance mapping
- T. Transmission Electron Microscopy
  - t1. Bright-field and/or dark-field imagery – TEM
  - t2. High-resolution (lattice-scale) TEM – HRTEM
  - t3. Selected area electron diffraction – SAED
  - t4. Chemical analysis by energy dispersive X-ray spectrometry – AEM
  - t5. Chemical structure/elemental valence by electron energy loss near-edge spectrometry – EELNES
- X. X-ray Methods
  - x1. Powder X-ray Diffraction – XRD
  - x2. Chemical structure/elemental valence by X-ray absorption near-edge spectrometry – XANES

- I. Ion Beam Methods
  - i1. Elemental/isotopic analysis by Secondary Ion Mass Spectrometry – SIMS
  - i2. Elemental/isotopic analysis by Time-of-flight SIMS – TOF SIMS
  - i3. Elemental/isotopic mapping by SIMS/TOFSIMS
- M. Mass Spectrometric Methods
  - mt1. Thermal ionization mass spectrometry – TIMS
  - mt2. Negative ion TIMS – NTIMS
  - mg1. Gas source mass spectrometry
  - mg2. Laser desorption, laser ionization –  $\mu\text{L}^2\text{MS}$
  - mg3. Laser desorption – LDMS
  - mg4. Time of Flight LDMS – TOF-LDMS
  - mg5. High-performance liquid chromatography/gas chromatography – HPLC/GCMS
  - mg6. Accelerator mass spectrometry – AMS
  - mg7. Pyrolysis – gas chromatography
- O. Optical Methods
  - o1. Petrographic microscopy
  - o2. Visible/NIR absorption spectroscopy
  - o3. Mid-infrared and thermal infrared absorption/emission spectroscopy
  - o4. Raman spectroscopy
    - o4a. Mineralogic mapping
  - o5. Cathodoluminescence spectroscopy
- SF. Scanning Force Microscopies
  - sf1. Atomic Force Microscopy - AFM
- MM. Magnetic Methods
  - mm1. Thermal demagnetization
  - mm2. Alternating field demagnetization
  - mm3. Magnetic susceptibility
  - mm4. Micro scanning SQUID imagery
- C. Chemical Methods
  - c1. High-performance liquid chromatography – HPLC
  - c2. Hydrothermal experiments
    - c2a. Cold-seal
    - c2b. Flow-through
  - c3. Inductively coupled plasma atomic emission spectroscopy for elemental composition – ICP-AES
- N. Nuclear Methods
  - n1. Instrumental neutron activation analysis – INAA
  - n2. Radiochemical neutron activation analysis – RNAA
  - n3. Mössbauer spectroscopy
  - n4. Nuclear track analysis
- B. Biological Methods
  - b1. Culturing on sterile media
  - b2. 16s RNA analysis
  - b3. DNA analysis
  - b4. Unspecified “biochemical methods”
  - b5. ? Polymerase chain reaction amplification of nucleic acids – PCR ?

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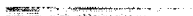
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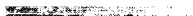
## APPENDIX D: BACKGROUND TUTORIALS



### Overview of Mars Sample Hazard Analysis (Requirements Workshop Series)

John D. Rummel  
Planetary Protection Officer  
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1



### SSB Recommendations for Mars Sample Return

- Samples returned from Mars should be contained and treated as though potentially hazardous until proven otherwise
- If sample containment cannot be verified en route to Earth, the sample and spacecraft should either be sterilized in space or not returned to Earth
- Integrity of sample containment should be maintained through reentry and transfer to a receiving facility
- Controlled distribution of unsterilized materials should only occur if analyses determine the sample not to contain a biological hazard
- Planetary protection measures adopted for the first sample return should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body

2

## Planning for Sample Hazard Analysis

Planetary Protection

### Protocol Development Workshops

- Major question: What are required steps to meet the NRC recommendation that, "rigorous analyses determine that the materials do not contain a biological hazard," and "returned samples should be considered potentially hazardous until they have been reasonably demonstrated to be nonhazardous"
- Plan: A series of workshops will be organized to assess the requirements for sample hazard testing and subsequent release, specify the tests necessary to show that a biological hazard is not present in the sample
- Action: Develop a recommended list of comprehensive tests, and their sequential order, that may be performed to fulfill the NRC recommendations in a manner acceptable to biomedical scientists and regulatory agencies

3

## Sample Hazard Analysis Assumptions

Planetary Protection

- The initial Mars Sample Return (MSR) may occur as early as October 2008.
- The missions will return samples from sites selected on basis of data to be returned from previous Mars Surveyor program missions.
- The samples will not be sterilized prior to return to Earth.
- Up to two separate sample return canisters (SRCs) will be returned to Earth in the initial mission. The SRCs will be opened only in a receiving facility.
- The amount of sample to be returned in each SRC is anticipated to be 500-1000 grams.
- The sample will likely be a mixture of types including rock cores, pebbles, soil, and atmospheric gases.
- The amount of sample used to determine if biohazards are present must be the minimum necessary.

4

## Sample Hazard Analysis Assumptions

(cont.)

Planetary Protection

- Samples must be handled and processed in such a way as to prevent terrestrial chemical or biological contamination.
- Strict containment of unsterilized samples will be maintained until quarantine testing for biohazards and life detection is accomplished. Sub-samples of selected returned materials may be allowed outside containment only if they are sterilized first.
- The receiving facility will have the capability to accomplish effective sterilization of sub-samples as needed.
- The receiving facility will be operational two years before samples are returned to Earth.
- The primary objective of the laboratory and protocols is to determine whether or not the returned samples constitute a threat to Earth's biosphere and population (not science study *per se*) and to contain them until this determination is made.

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## Workshop Plan

Planetary Protection

- Workshop I
  - » March 20-22, 2000, Bethesda, Maryland USA
  - » Objective: Establish the context, overall approach and product(s) of the workshop series; outline a preliminary, comprehensive, beginning-to-end scenario for a Mars sample handling protocol and timeline to determine if the samples contain a biological hazard.
- Workshop II a/b
  - » April-September 2000, East Coast USA
  - » Objective: Develop MSR PP life detection approaches (with NRC) and integrate with biohazard determination protocols and timeline from Workshop I (will be a two-phase activity). Specify in detail the preferred methodologies for biohazard determination and life detection that will comprise the protocol.

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## Workshop Plan (cont.)

Planetary Protection

- Workshop III
  - » October 2000-January 2001, East Coast or California USA
  - » Objectives:
    - Specify detailed requirements to be met by any protocol
    - Delineate acceptable MSR sample hazard determination and analysis principals and known protocols, and the maintenance and oversight process for modification/updating of protocol by sample handling project
    - Integrate and finalize sample handling requirements and methodologies into an initial protocol on which to base facility cost/sizing projections; outline final report findings and recommendations.

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## Questions/Issues: Workshop 1

Planetary Protection

- What types/categories of tests (biohazard determination, life detection) should be performed upon the samples? What scientific controls should be implemented? What preliminary characterization information is required for these tests to be implemented?
  - » Identify amounts of sample needed for these tests.
- How will representative sub-samples for all tests be selected?
- How will the nature of the sample (i.e., rocks, soil, cores, etc.) affect the tests chosen?
- In what sequence shall the relevant testing be performed?
- What tests can be performed on sterilized samples outside of containment?

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## Questions/Issues: Workshop 1 (cont.)

PLANETARY PROTECTION 

- What is the preferred method of sterilization to preserve information content of samples?
- What is the range of relevant test results and interpretations that might cause concern?
- What are the criteria for release of samples from containment?
- Assess the pros and cons of using multiple containment facilities to determine if the samples contain a biological hazard.

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## Questions/Issues: Workshop 2

PLANETARY PROTECTION 

- In what sequence will the specific characterization, biohazard determination, and life detection analyses be performed?
- What are the necessary, sufficient, and relevant biohazard determination and life detection tests to be performed?
- What are the various possible interpretations of results from the suite of biohazard determination and life detection analyses?
- Assess the extent to which the detailed tests meet the objectives of other interested parties (e.g., regulatory agencies, international partners, etc.)

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## Questions/Issues: Workshop 3

PLANETARY PROTECTION 

- Integrate the detailed methodologies for biohazard determination and life detection into a recommended protocol and timeline.
- Assess how the recommended analyses will satisfy the criteria for release of samples from containment.
- How will advances in methods/technologies in the coming years be incorporated into the recommended protocol? How will the protocol be amended in the future up to the receipt of samples? How will this process be overseen/reviewed by Planetary Protection?
- What considerations of facilities, equipment, and personnel are important for implementing the recommended protocol?
- Develop outline of findings and recommendations for final report.

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## Planning for Sample Hazard Analysis

WORKSHOP 1: PLANNING FOR SAMPLE HAZARD ANALYSIS

Planetary Protection

- Organizing committee, Chaired by NASA Planetary Protection Officer (with CNES participation)
- Senior-Level Oversight and Review Panel (~25 people) to advise the organizing committee on the planning, organization, participants, and conduct of the workshops (US and France)
  - » Chosen for their abilities to address key scientific, biohazard evaluation and quarantine protocol issues associated with handling, characterizing, testing, and judging whether returned sample materials are in any way biohazardous, and when and whether they may be certified for controlled distribution outside containment and quarantine
  - » Will provide peer review of the protocol, prior to its release for external review by appropriate groups outside of NASA
- Workshop participants (by invitation)

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## Workshop Products

WORKSHOP 2: WORKSHOP PRODUCTS

Planetary Protection

- Individual Workshops:
  - » Summary of material analyzed (advance reading, handouts, subgroup reports, etc.)
  - » Interim report of findings and recommendations prior to next workshop
  - » Briefing package
- Final Workshop Series:
  - » Final report of findings and recommendations, reviewed by Oversight and Review Committee
  - » Briefing package suitable for presentation to advisory groups, regulatory agencies, scientific meetings, etc.
  - » Recommendations in a form suitable for use as input for possible future announcements of opportunity soliciting proposals for Mars sample handling participants/capabilities.

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## Planning for Sample Hazard Analysis

WORKSHOP 3: POST-WORKSHOP TASKS

Planetary Protection

- Post-Workshop Tasks
  - » Preparation of overall report and protocol details
  - » Review by Oversight and Review Panel and revisions
  - » Submit final document
  - » Endorsement by NASA Advisory Council / Planetary Protection Advisory Committee; Parallel review by CNES, etc.
  - » Dissemination of report to relevant audience(s) or Agencies
  - » Approval by other Agencies, and availability for use in planning for activities in the Mars Receiving Facility, etc.

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# Planetary Protection Overview

Dr. John D. Rummel  
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Office of Space Science

3/20/00

1

## Planetary Protection in NASA

### Current Focus (NPD 8020.7)

- Preserve biological and organic conditions for future exploration
- Protect the Earth from potential extraterrestrial contamination

### Scope and Applicability

- All NASA missions to other planetary bodies or that return samples to Earth
- Non-NASA missions with NASA participation

### Philosophy

- Planetary protection is a “way of life” in solar system exploration
  - It is integral to the endeavor, not an add-on or afterthought
  - NASA must police itself

2



## International Agreement on Planetary Protection

Planetary Protection 

### Article IX of the Outer Space Treaty of 1967:

- "...parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose..."

"Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies."  
(entered into force, October 10, 1967)

3

## Current Planetary Protection Activities

Planetary Protection 

### Overall

- Maintenance of a NASA policy, consistent with international agreements
- Planetary protection policy management in OSS, with Field Center support
- Advice from internal and external advisory groups (NRC, NAC/Planetary Protection Task Force)
- Technology research and standards development in bioload characterization
- Technology research and development in bioload reduction/sterilization

### Forward contamination

- Research on the potential for Earth life to exist on other bodies
- Improved strategies for planetary navigation and collision avoidance
- Improved procedures for sterile spacecraft assembly, cleaning and/or sterilization

### Back contamination

- Development of sample transfer and container sealing technologies for Earth return
- Improvement in sample return landing target assessment and navigation strategy
- Planning for sample hazard determination requirements and procedures, safety certification
- (liaison to NEO Program Office for compositional data on small bodies)
- Facility planning for sample recovery system, quarantine, and long-term curation of returned samples

4



## Mars Sample Handling Protocol Workshop series

French Participation in Mars Sample Return  
(and MARS exploration)

**Jean-Louis Counil**



### Mars Exploration Program French Contribution



- High level contribution to the first MARS Sample Return mission
- Leadership of the European Netlander project:
  - First Geophysical Network on Mars (4 stations)

(around 500 MS)

+

- Payload Instruments on the ESA-mission MARS-Express
- Contribution to US Micro-missions
- Instruments on Landers (PALOMA, Ma-FLUX)
- Co-Is

Jean-Louis COUNIL, March 20, 2000




### The NETLANDER Mission




- Internal Structure
- Surface and sub-surface geophysics
- Global meteorology and Climate
- Four identical surface probes, each with:
  - Seismometer
  - Meteorological package
  - Panoramic Camera
  - Geodesy experiment
  - Magnetometer
  - Ground water detector (radar)
  - Ionospheric package



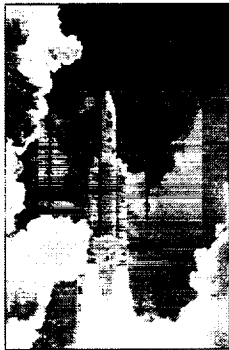
Jean-Louis COUNIL, March 20, 2000




## Mars Sample Return Mission


4


- Dual Ariane V launch for the CNES Orbiter and an US lander




- Mars Sample Return Orbiter  
(NETLANDERs as auxiliary payload)



Jean-Louis COUNIL, March 20, 2000




## Mars Sample Return Program French views



5

- France to contribute to all phases of the sample return mission, in particular
  - preparation phase
    - architecture
    - site selection
    - handling protocol
  - analysis phase
    - quarantine protocol definition and implementation
    - science analyses
    - curation
- France to be associated to main decisional aspects of the program:
  - authorization to land the samples
  - authorization to end out the quarantine
- A specific Public Outreach Program is being implemented.

Jean-Louis COUNIL, March 20, 2000




## Mars Sample Return Program On-going activities



6

- Information to all relevant communities through workshops
- Scientific Preparatory Program under consolidation
  - AO released in February
  - Deadline for responses: April 15
  - Start of funding/activities: Sept 00.
- Strong mobilization of the French Community :
  - 160 scientists from 72 research teams have attended the Jan 11-12 workshop on : "Scientific Analysis of the Martian samples"
  - Over 70 LOI received !
  - A large variety of communities involved:
    - planetologists, geophysicists, geo-chemists, mineralogists
    - biologists and astrobiologists, chemists

Jean-Louis COUNIL, March 20, 2000




### Quarantine activities French expertise




- Many teams have declared their interest in contributing to:
  - scientific activity
  - technical activity

based on their high level expertise.




P4 Mérieux Laboratory, Lyon




Use of the former French  
Nuclear missiles Facility


Now declassified for scientific uses



Jean-Louis COUNIL, March 20, 2000



### Mars Sample Return Program French Organization



- French MARS Program managed at a national interagency level by a MARS Steering committee, with representative of:
  - CNES
  - CNRS
  - Ministry of Education, Research and Technology
    - Direction of Research
    - Direction of Technology
- Two committees have been set that report to the MARS Steering Committee
  - Scientific Committee for Sample Analysis
  - Planetary Protection Committee

8

Jean-Louis COUNIL, March 20, 2000

**Presentation to Mars  
Sample Handling Workshop  
March 20, 2000**

Kenneth Nealson  
Astrobiology Group, JPL  
Faculty Associate, Caltech

Summary of 1992 and 1997  
NRC (Space Studies Board)  
Task Group Reports

Dr. Nealson served as chair of both of these NRC task groups. The two reports are published by NRC Press. A short summary of these is outlined below in order to stimulate discussion

1

**Biological Contamination of Mars  
Issues and Recommendations**

**1992 Report**

Written in 1990/91

Published in 1992

Adopted by COSPAR in 1994

Kenneth Nealson, U. Wisconsin, Chair  
John Baross, U. Washington - Microbiology  
Michael Carr, USGS - Mars Science  
Robert Pepin, Univ. Minnesota - Mars Science  
Thomas Schmidt, Mich. State U. - Microbiology  
Jodi Shann, U. Cincinnati - Microbiology  
J. Robi Vestal, U. Cincinnati - Microbiology  
David C. White, U. Tenn. - Microbiology  
James Ferris, RPI - Origin of Life  
Norman Pace, U. Colorado - Evolutionary Biology

2

**Biological Contamination of Mars  
Issues and Recommendations**

**Charge to the Task Group:**

1. Reassessment of Planetary Protection
  - a. Assess status of previous reports and their implementation
  - b. Describe current state of knowledge regarding planetary protection
  - c. Recommend research needed to address issues relevant to current needs in Planetary Protection

3

**Brief Overview -1-**

The task group responded with a document that generally supported the previous statements regarding planetary protection, but in addition, made 10 recommendations in several key areas.

The assessment of the 1978 report was generally positive with the exception of the use of the term  $P_g$ , the probability of growth of a given organism.  $P_g$  was regarded as a non-useful term.

It was also stated that the probability of Earthly life prospering as contaminant growth on Mars was not an issue.

4

## Brief Overview -2-

The more relevant issue, it was felt was the issue of potential contamination of potential life detection experiments (science protection).

It was this thinking that lead to 10 recommendations in the categories defined below:

1. Bioburden Assessment (2)
2. Cleaning & Sterilization (2)
3. Science Recommendations (2)
4. Public Engagement (3)
5. Implementation (1)

5

## Summary of Recommendations Bioburden Assessment

1. Efforts should be made to adopt current molecular analytical methods for use in bioburden assessment and inventory procedures for spacecraft assembly and launch for future missions, and also to develop new methods for the same purposes.

6. Viking protocols for assessment of spacecraft bioloads should be upgraded to include state-of-the-art methods for the determination of bioload.

6

## Summary of Recommendations- Cleaning and Sterilization

2. Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Methods are not specified.

3. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures – such as clean-room assembly and cleaning of all components – for bioload reduction but such spacecraft need not be sterilized.

7

## Summary of Recommendations Science Issues

4. A sequence of unpiloted missions to Mars should be undertaken well in advance of a piloted mission.

5. A broad spectrum of martian sites should be examined with emphasis on measurements that provide data most likely to contribute to a better understanding of the probability of life on Mars and where best to go to be able to detect it.

8

### Summary of Recommendations Public Engagement and Implementation

7. Inform the public !!
8. Assess legal issues and limits !!
9. Obtain international agreements
10. NASA should redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the above recommendations.

9

### Probability of Contamination

1978 Report –

Probability of Contamination ( $P_c$ )  
should be less than 1 in  $10^8$

Included  $P_g$  (P of growth), which required knowledge of physical and chemical properties of planet, along with knowledge of limits of life of earthly organisms.

Committee concluded that this was not a useful parameter

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### Other Notes

Committee Report has several valuable appendices concerning:

Historical issues in PP  
Properties of organisms  
Properties of Mars  
Mission category requirements  
Summary of Viking data as they relate to probability of growth of earthly life on Mars  
Discussion of  $P_g$  as applied to Mars and other solar system bodies

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### Issues in Sample Return

Mars Sample Return:  
Issues & Recommendations (1997)

Kenneth Nealson, U. Wisconsin (JPL) Chair  
Russell Doolittle, UCSD, Evolutionary Biol.  
Norman Pace, U. Colorado, Evol. Biol.  
Andrew Knoll, Harvard, Paleobiology  
Jeanne Poindexter, Columbia, Microbiology  
Bruce Jakosky, U. Colorado, Planetary Sci.  
Michael Carr, USGS, Mars Science  
J. W. Schopf, UCLA, Paleobiology  
Ben Clark, LMI, Space Engineering  
Ed Korwek, Regulatory Law  
Margaret Race, SETI, Ecology  
Anna-Louise Reysenbach, Molecular Biology  
Todd Stevens, DOE, Microbiology

Available on the Web:  
[www.nas.edu/ssb/mrsmenu.html](http://www.nas.edu/ssb/mrsmenu.html)

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### Issues in Sample Return Charge to the Task Group

The Task Group was asked to address the following concerns:

1. The potential for a living entity to be included in a sample to be returned from another solar system body, in particular Mars;
2. The scientific investigations that should be conducted to reduce uncertainty in the above assessment;
3. The potential for large-scale effects on the environment resulting from the release of any returned entity;
4. The status of technological measures that could be taken on a mission to prevent the unintended release of a returned sample into Earth's biosphere; and,
5. Criteria for controlled distribution of sample material, taking note of the anticipated regulatory framework.

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### Issues in Sample Return Brief Overview

The task group reported findings that led to recommendations in three general areas:

1. While Mars is a very harsh environment for life, it is plausible that "oases" for life do exist in the subsurface or yet-to-be-discovered environments.
2. Contamination of Earth by martian organisms is extremely unlikely, but the risk can not be shown to be zero.
3. Uncertainties with regard to martian life can be reduced through a program of research, but until proven otherwise, each sample should be assumed to viable exogenous biological entities.

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### Issues in Sample Return Brief Overview -2-

Recommendations were made in three general areas:

1. Sample Return and Control
  - Sterilization and Containment
  - Sample integrity
  - Sample distribution
  - Relaxation of PP with time
2. Sample Evaluation
3. Program Oversight
  - Oversight panel is needed
  - NASA structure is needed
  - Public needs to be informed

15

### Issues in Sample Return Findings -1-

Potential for returning ET organism:

VERY LOW – Mars surface is likely sterile

BUT NOT ZERO –

Oases may exist  
Terrestrial life can be very extreme

Potential for large-scale effects from Ets

VERY LOW – many reasons for this statement, outlined in the report

BUT NOT ZERO – must be treated with caution

16



### Issues in Sample Return Findings -2-

Research that could reduce uncertainty:

1. Task group endorses NASA's "Exobiological Strategy for Mars Exploration"
2. Other research could include:
  - study of terrestrial extremophiles
  - further study of Mars meteorites
3. Hard to prove a negative research will enhance scientific utility but will probably not alter PP requirements

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### Issues in Sample Return Findings -3-

Technical measures to reduce risk

Areas where R & D is needed:

1. Life detection at low levels
2. Mars-relevant sterilization technology
3. Effective in-flight containment
4. Verification of containment

18

### Issues in Sample Return Recommendations -1-

1. Sample Return and Control
  - A. If sample containment can't be verified en route to Earth, the sample should either be sterilized in space or not returned
  - B. Integrity of containment should be maintained through reentry and transfer of sample to an appropriate receiving facility
  - C. Controlled distribution of unsterilized materials should occur only after it is declared safe. If samples are removed prior to this, they should be sterilized.
  - D. PP measures adopted for the first MSR should not be relaxed for subsequent missions without thorough scientific review

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## Issues in Sample Return Recommendations -2-

### 2. Sample Evaluation

A. A research facility for receiving, containing, and processing returned samples should be established as soon as possible once serious planning for a MSR mission has begun. At a minimum, the facility should be operational at least two years prior to launch. The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization. An advisory panel of scientists should be constituted with oversight responsibilities for the facility.

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## Issues in Sample Return Recommendations -3-

### 3. Program Oversight



A. A panel of experts, including representatives of relevant governmental and scientific bodies should be established (ASAP) to coordinate regulatory responsibilities and to advise NASA on implementation of PP. The panel should be in place one year prior to establishment of the sample receiving facility.

B. An administrative structure should be established within NASA to verify and certify adherence to PP requirements at all mission stages.

C. Throughout any sample return program, the public should be openly informed of plans, activities, results, and associated issues

21

1








# Mars Sample Return Mission Design

**Robert Gershman**  
Planetary Protection Manager  
Mars Sample Return Project


Presented to  
Mars Sample Handling Protocol Workshop  
Bethesda, MD

20 March 2000

NOTE: MSR Project is being rescoped and rescheduled. Calendar information shown is obsolete and provided for illustration purposes only.










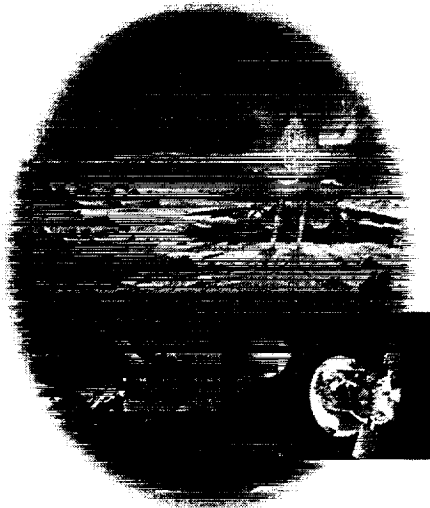
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*Mars Sample Return Mission Design*

## Introduction



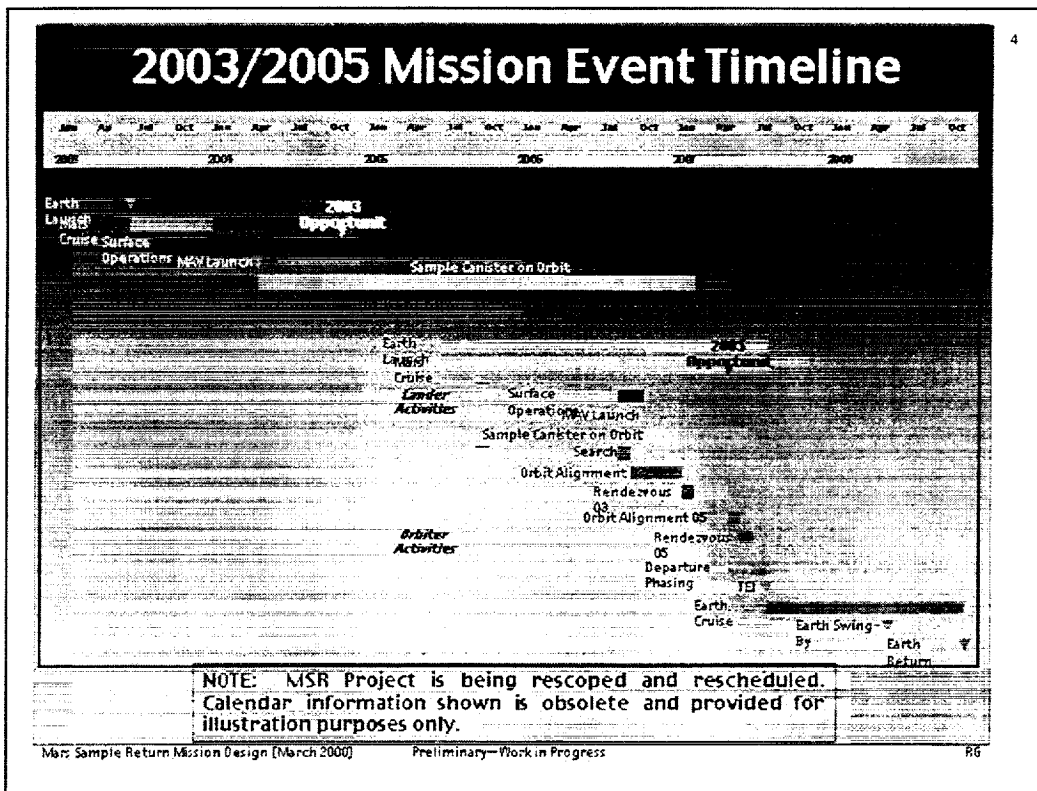
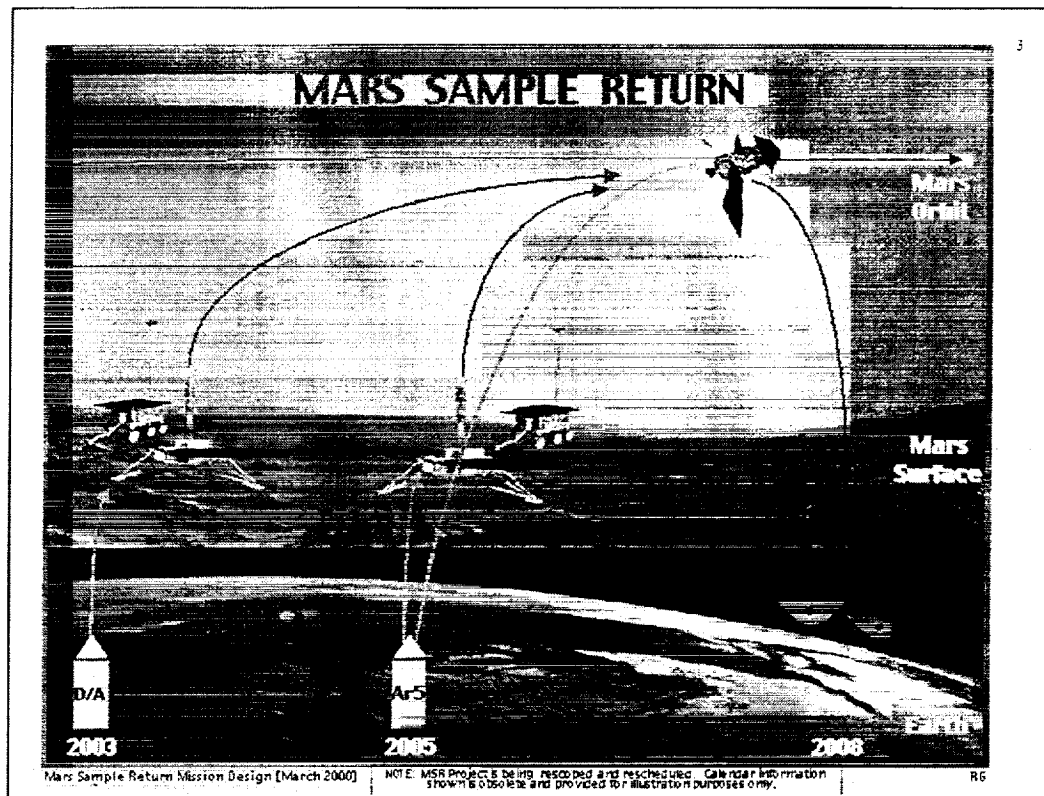


- **Topics**
  - Mission Overview (03/05)
  - Redesign and Reschedule Options
  - Sample Transfer Chain
  - Bioburden Control
- **Caveat**
  - Program Plan is evolving rapidly

Mars Sample Return Mission Design [March 2000]

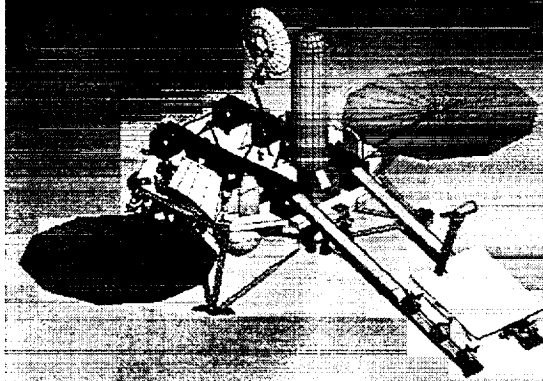
Preliminary—Work in Progress

R6



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## Sample Transfer Chain



- Sample transfer
- Sample cache on rover
- Sample transfer to Mars Ascent Vehicle (MAV)
- Lander-based sampler
  - Expecting ASI supplied drill
- Orbiting Sample (OS)
  - Sealed sample container
  - Solar power radio beacon
- Orbiter capture and transfer equipment
  - Components across all flight elements
  - Focus of planetary protection cleaning and sealing

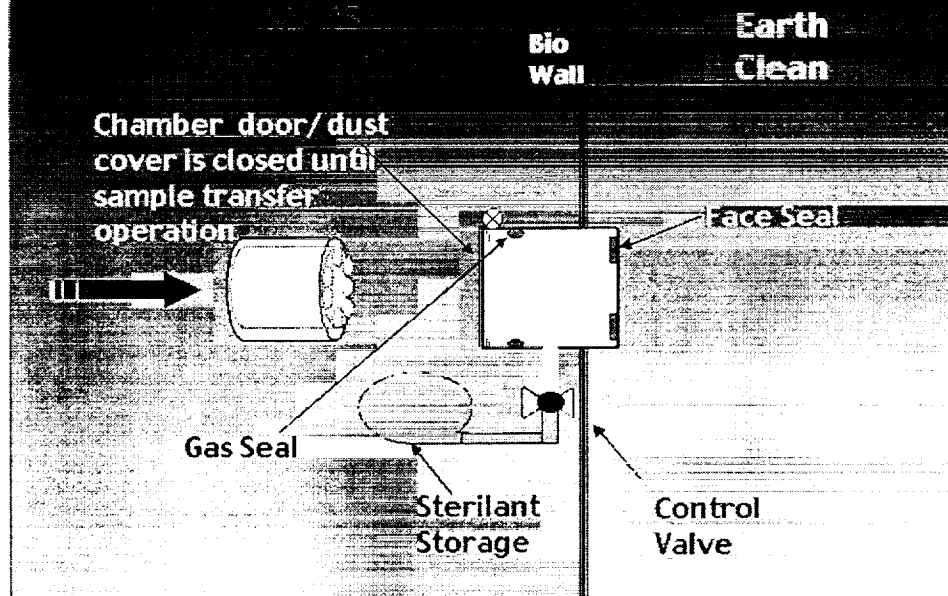
Mars Sample Return Mission Design [March 2000]

Preliminary—Work in Progress

R6

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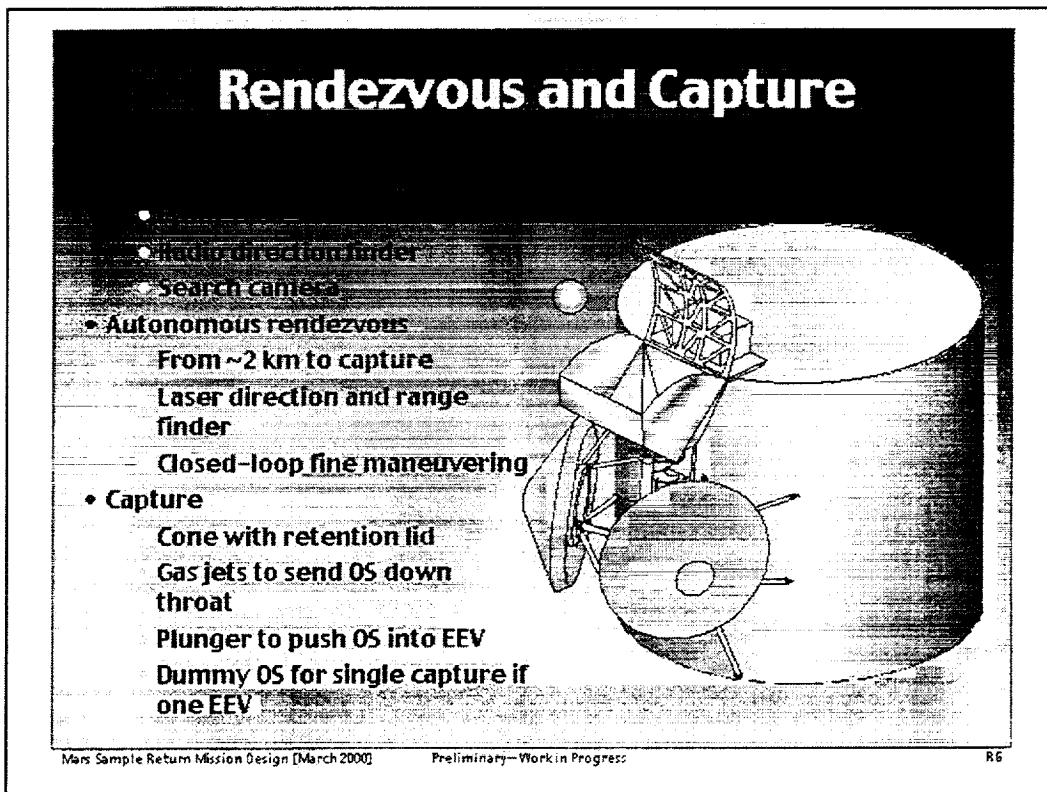
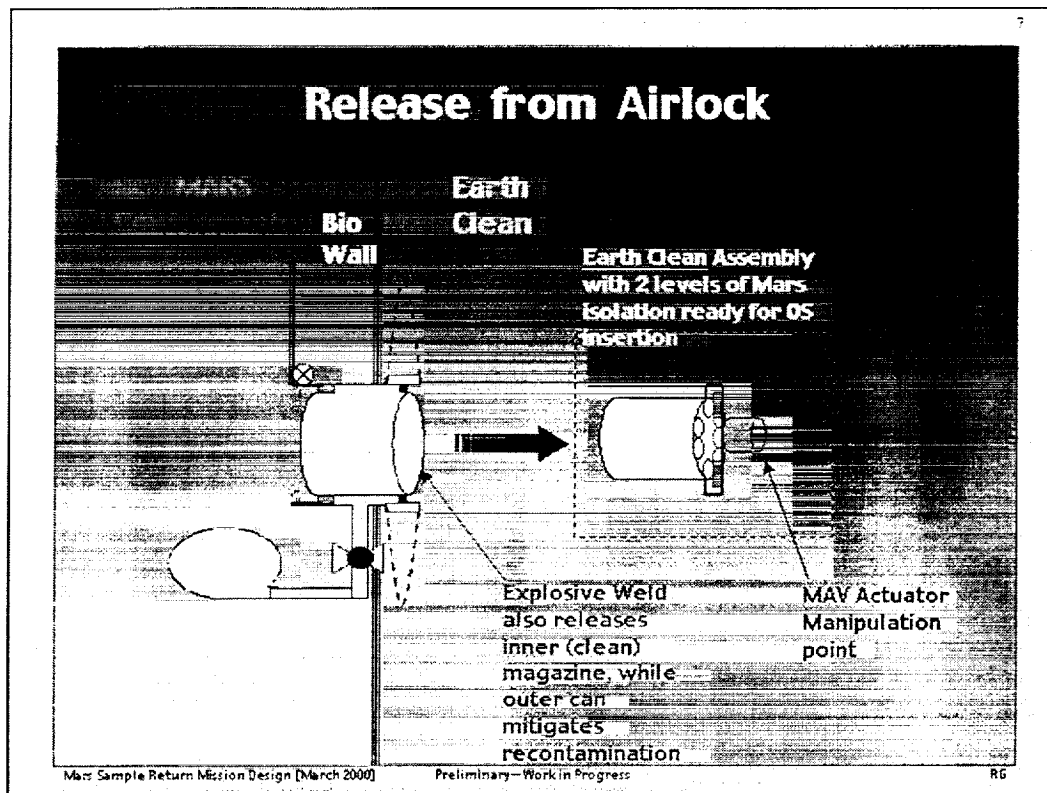
## Sample Magazine Meets “Airlock”

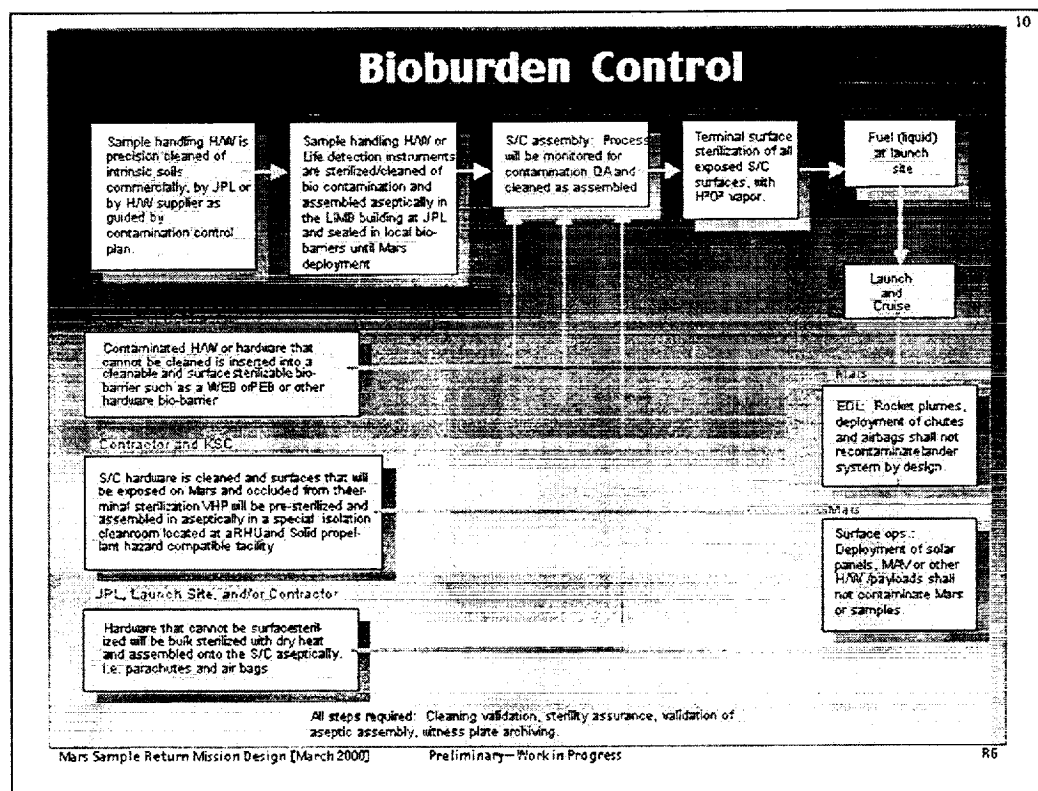
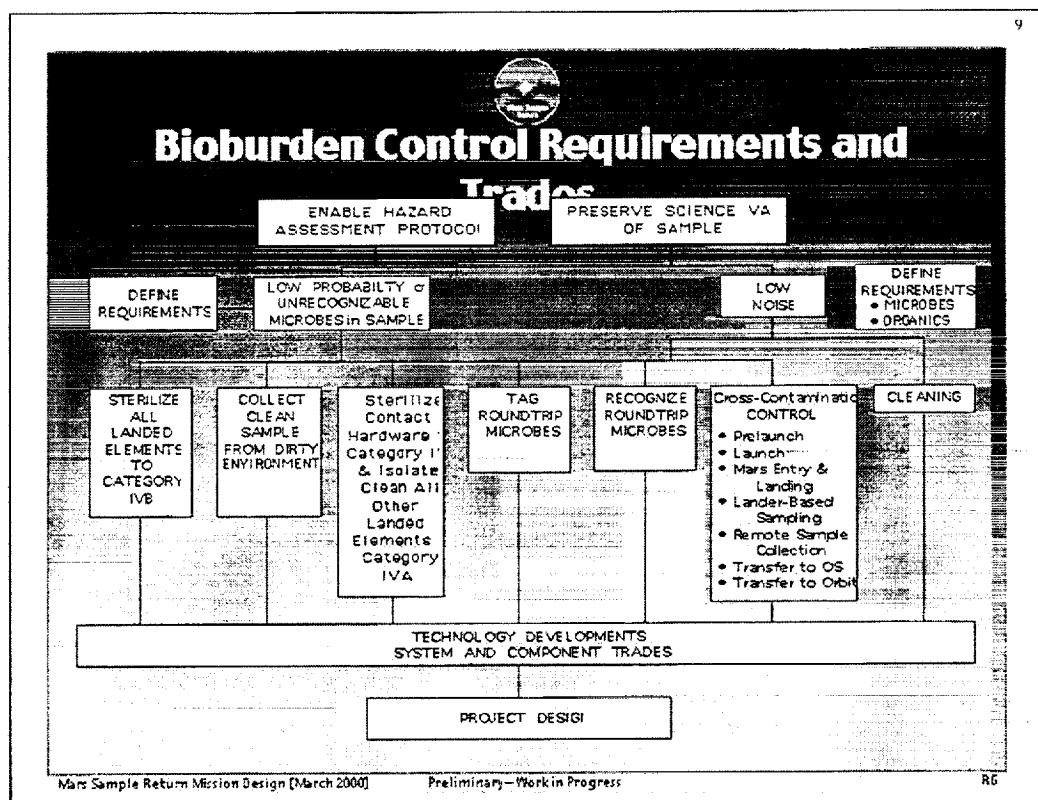


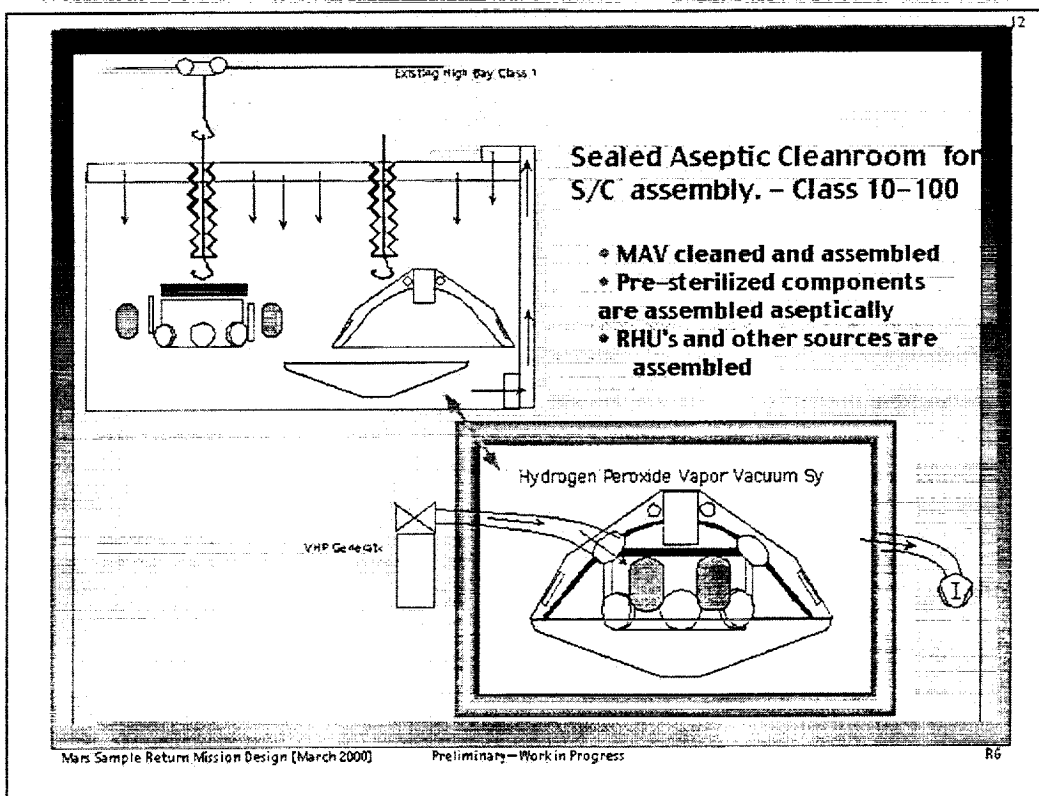
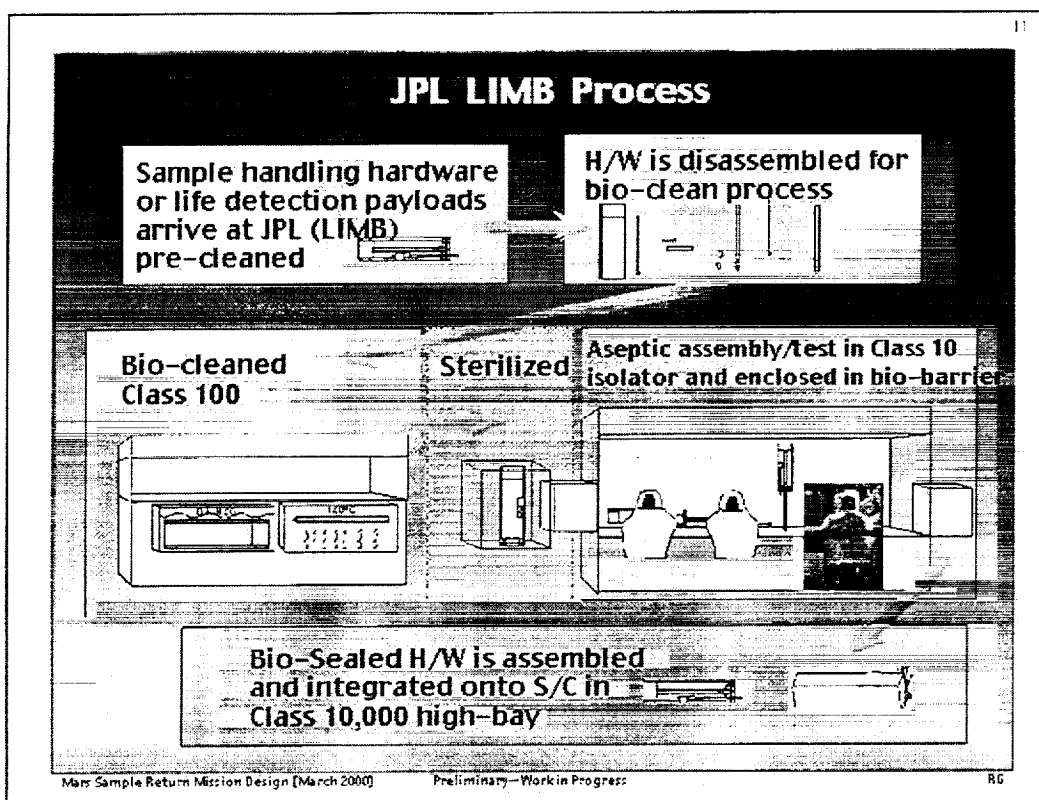
Mars Sample Return Mission Design [March 2000]

Preliminary—Work in Progress



R6










**Mars Sample Return Mission Design**  
**Project Status Summary**



- Excellent progress has been made on MAV and Sample Transfer Chain development.
- Work on development of robust lander concepts (not 98/01 heritage) is well under way.
- MSR Orbiter partnership with CNES is fully intact.
- Development of trades among options for cleaning/sterilizing the spacecraft will receive a lot of attention in the next year.

Mars Sample Return Mission Design [March 2000] Preliminary—Work in Progress R6

## Options in Extraterrestrial Sample Handling and Study

Dimitri A. Papanastassiou  
JPL

Mars Sample Handling Protocol Workshop  
March 20, 2000

1

### First Order Concepts

- Sample preservation, hazard assessment, and handling are important service functions
- Preliminary examination of samples is necessary for sample hazard assessment and for sample allocations
- Clean facilities and clean sample handling are required
- Conflicts, cross contamination issues will be present and need to be resolved
- Extensive experience is available for extraterrestrial samples and must be sought and applied
- Extensive experience is available in studies of pathogenicity and must be sought and applied as necessary
- Advisory and oversight structures must be in place

2

### Analytical and Technical Aspects

- Low contamination
- High sensitivity
- Trained personnel
- Proper tools
- All translate into time to develop techniques and gain experience

3

### Analytical and Technical Aspects – History

Limited samples from the Soviet Union: Luna 24

- A total of 3 g allocated to U. S. investigators
- Conference attracting 105 investigators, 67 papers; Proceedings published, 750 pages
- Example: petrology, mineralogy, chemistry, irradiation history, internal isochron, crystallization ages (on 97 mg of chips of a gabbro)
- MESSAGE: high sensitivity required

4

### Analytical and Technical Aspects – Apollo 11 History

- Most preconceptions about the Moon were wrong
- Opportunity for extensive instrumental and analytical developments
  - Some lack of preparation
    - in precision, level of contamination
    - Several investigations were impossible for several years (e. g., U-Pb dating of lunar basalts)
  - Need adaptive strategy for development of techniques
- Biohazard testing: ~700 g used (just Apollo 11)
- Apollo 11 Proceedings: ~3200 pages

5

### Existing and Planned Sample Collections

- Lunar Samples: Lunar Curatorial Facility, JSC
  - <http://www-curator.jsc.nasa.gov/lunar/lunar.htm>
- Antarctic Meteorites: Antarctic Meteorite Laboratory, JSC
  - <http://www-curator.jsc.nasa.gov/antmet/antmet.htm>
- Cosmic Dust: Cosmic Dust Laboratory, JSC
  - <http://www-curator.jsc.nasa.gov/dust/dust.htm>
- Stardust: Planned to be handled in Cosmic Dust Laboratory

6

### Existing and Planned Sample Collections

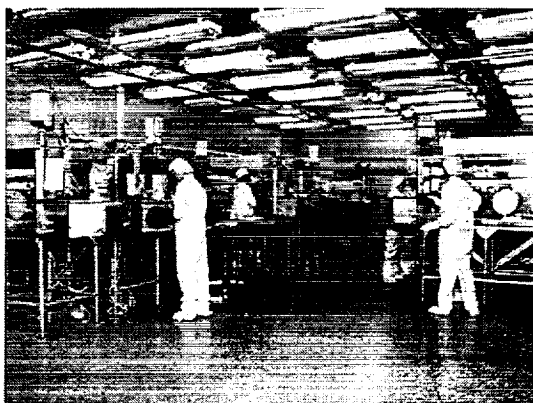
- Genesis: Clean lab constructed in a support area of the LCF
  - Recently certified as a Class 1 room
- Muses-CN: Samples to be curated in Japan
  - With US investigators participating
  - After one year the US allocation will be handled at JSC
- Comet Nucleus SR:
  - Planning stages: Requirement for very cold temperatures
- Mars Sample Return: Facilities and sample handling under active consideration

7

### Lunar Curatorial Facility

- Protocols and practices and historical perspective to be presented by J. Allton, JSC
- But
- Original Lunar Receiving Laboratory (LRL) designed for quarantine and hazard assessment, with limited facilities for handling, storing, and examining samples
  - Original LRL cost became a problem for securing the funding for the present facility

8



9

### Antarctic Meteorite Laboratory, JSC

- Samples received frozen
- Samples allowed to thaw and dry in stainless steel cabinets purged with dry nitrogen
- Samples described, sampled for thin section
- Descriptions published in Antarctic Meteorite Newsletter (since June, 1978), many in five Smithsonian Contr. Earth Sci., and on line
- Sample allocations through Meteorite Working Group, twice per year, to research scientists
- Consortium arrangements for unique samples

10



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### Cosmic Dust Laboratory, JSC

- Class 10 clean room, special design to avoid high air velocities
- Maintain sample collectors (Lexan with thin film of silicone oil) in dry nitrogen, until samples needed
- Procedures for optimum use of particles; specialized handling, cleaving, etc.
- Catalogue particles, make particles available
- Support visiting scientists in particle handling and transfer of particles to appropriate substrates
- Also: best record of terrestrial and space debris

12



13

### Stardust (Discovery Mission)

- Collectors and samples planned to be handled in Cosmic Dust Laboratory, JSC
- Experience with extracting samples from aerogel; further developments in progress by PI and Science Team
- Need to control aerogel dispersion

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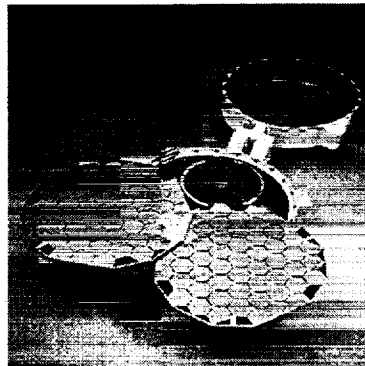
### Genesis (Discovery Mission)

- Genesis Lab: Clean lab constructed in a previous support area of the LCF (1st floor)
  - Design with help from PI and CAPTEM, Facilities Subcommittee
  - Change, equipment transfer stations
  - Cleaning station (room), with ultra pure water
  - Space collector assembly and handling lab
    - Recently certified as a Class 1 room
  - Protocols being established by PI and science team, in collaboration with facilities personnel
- General allocation of samples, through CAPTEM, as soon as feasible

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### Mars Samples

- Planetary protection and hazard assessment required, in contrast to other sample collections
- Lunar experience
  - Complicated by considerations for astronauts
  - Quarantine was required for Apollo 11, Apollo 12, Apollo 14, and for samples from later missions from “new environments” (e.g., Apollo 15 deep drill)
  - These requirements extended well past demonstrated hazard assessment need
  - Lesson learned: need to define the requirements for hazard assessment and to devise a protocol that addresses the requirements

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### Mars Samples - Contamination

- Mission design: sample acquisition
  - lander-based:
  - mobility (rover) based
- Contamination
  - inorganic
  - organic
  - biologic
- Sample conditions
  - sample isolation; containment; atmospheric sample
  - temperature
  - impact effects

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### Mars Samples - Contamination

- Materials choices
  - choices extend to containers as well as sampling tools (drill bits), lubricants, adhesives, brazing alloys
  - rocket exhaust
  - vacuum, temperature behavior/outgassing
    - final choice: engineering requirements; selection possible based on science requirements
    - testing required
  - Coupons: record materials and conditions

20

### Mars Samples - Contamination

- Cleaning techniques
  - Level of contamination
  - Material compatibility
  - Technology development needed

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## Mars Samples - Contamination

### Reality Check

- Ultra clean sample collection required, in anticipation of the more advanced and sensitive analytical techniques when samples are returned N years from today
- It is a practical impossibility for processes to be cleaner today than the current state-of-the-art sensitivity and blank contamination levels

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## Most Critical Issues

- Address needed investigations
- Minimize contamination (current state-of-the-art)
- Improve sensitivity
- Improve laboratory infrastructure
- Provide preliminary characterization of samples
- Provide hazard assessment
  - Define hazard and needed response options
  - Define Preliminary Examination of samples to help identify hazard and choose response option
  - Define consistent sample distribution options

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## Most Critical Issues ( cont.)

- Provide peer review for proposals for work on Mars samples
- Provide a single, international committee for sample allocation to approved PIs

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### Sample Handling Concepts

- Sample collection, receiving, curation, preliminary examination, hazard assessment, and allocation are service functions
- Concept of Consortium Investigations
- Distinction between destructive and non-destructive techniques
  - “non-destructive” requires definition, in relation to contamination preventing subsequent uses of sample
  - destructive analyses

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### Mars Sample Facilities

- Mars Receiving Facility
  - Clean, sterile, and cleanable facility, providing BSL-4 level of bio-containment
  - Must preserve inorganic, organic, and biological integrity of samples
  - A new concept: no such facility currently exists
  - Feasible
  - Must include separate laboratories for preliminary examination and hazard assessment

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### Mars Sample Facilities

- Mars Curation Facility
  - Must preserve inorganic, organic, and biological integrity of samples
  - Must provide facilities for subdivision, documentation, allocation, long term storage
  - Must be able to host investigators

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### Protocol Development - Parting Thoughts

- Address sensitivity of techniques and instrumentation
- Address sample preservation and cross contamination
  - Avoid markers/tracers as distinct from witness plates
- Address preliminary examination of samples
  - knowing rock type and chemistry is important for hazard assessment
  - early information, even on sterilized samples
- Maintain witness plates to address false positives

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## Mars Sample Handling and Requirements Panel (MSHARP) Report Summary

Mars Sample Handling Protocol Workshop  
Bethesda, MD

Mar. 20, 2000

**Donald L. DeVincenzi**  
NASA Ames Research Center



## MSHARP Charter

- Chartered Fall 1997 by Associate Administrator for Space Science at NASA HQ
- Recommend requirements in three areas
  - + Sample collection and transport back to Earth
  - + Certification of samples as non-hazardous
  - + Sample receiving, curation and distribution
- Do not debate implementation details
- Use 1997 NRC report (Nealson) as a guide

2



## MSHARP Membership

- M. Carr, Chair, USGS Menlo Park, CA (geologist)
- J. Bada, Scripps Institution (organic geochemist)
- D. Bogard, JSC (geochemist)
- B. Clark, Lockheed Martin (biogeochemist)
- M. Drake, U. Arizona (geochemist)
- D. DeVincenzi, ARC (planetary protection)
- D. McCleese, JPL (atmospheric physics)
- K. Nealson, JPL (microbiologist)
- J. Papike U. New Mexico (geochemist)
- M. Race, SETI Institute (environmental sciences)
- D. Stahl, Northwestern (microbiologist)

3



## Mars Sample Return (MSR) General Considerations

- Goals of the MSR mission/system
  - ✦ Return samples to Earth unaltered and free of contamination
  - ✦ Prevent uncontrolled release of Mars materials into environment
  - ✦ Maximize science return from the samples
- Drivers of Mars sample handling
  - ✦ Traditional biosafety and planetary protection concerns
  - ✦ Sample protection and science considerations
- Mars science issues
  - ✦ Search for past or present life is primary objective of Mars program
  - ✦ Many science issues resolved only with variety of returned samples
  - ✦ Present Mars surface is very hostile to life
  - ✦ Presence of life and risk of harm is low but not zero

4



## Sample Acquisition and Containment

- Assume returned samples are hazardous until proven otherwise
  - ✦ Place samples in sealed containers on Mars
  - ✦ Containers to be opened only in BSL-4 containment facility
  - ✦ No samples leave containment unless sterilized or proven harmless
- Return options considered
  - ✦ Return to International Space Station is impractical and risky
  - ✦ Return of sterilized sample is difficult and scientifically undesirable
  - ✦ Return pristine samples to Earth containment facility is best
- Nature of samples anticipated
  - ✦ Rover-acquired rock cores
  - ✦ Soil samples
  - ✦ Atmospheric sample

5



## Control of Forward Contamination of Samples

- Contamination of samples with terrestrial materials is serious problem
  - ✦ It can compromise science results from samples
  - ✦ False positives can confuse biohazard interpretation, sample release
- Mission procedures recommended
  - ✦ All landed components cleaned according to current standards
  - ✦ All components touching samples to be sterilized and cleaned
  - ✦ Use tracers, witness plates, assays to identify contaminants
- Related concern is to minimize sample alterations
  - ✦ Maintain samples at temperatures no higher than 240 K during return

6



## Control of Back Contamination of Earth

- Mission design considerations
  - Seal sample canister on martian surface
  - Verify integrity of seal before leaving Mars environment
  - Transfer sample to return vehicle in way that precludes transfer of uncontained materials
  - Design against failure rather than monitoring, contingency plans
    - Incorporate multiple seals, interleaved filters

7



## Control of Back Contamination of Earth (continued)

- Sample receiving facility (SRF) considerations
  - Sample canister returned to containment facility equivalent to BSL-4
  - Facility must meet cleanliness standards for extraterrestrial samples
  - New facility is needed to meet both these requirements at same time
  - SRF is service facility for community rather than research facility
    - Research should be done by community through NRA process
    - Determination of hazard is the service role of the SRF
      - Early inventory of samples
      - Preliminary hazard assessment and life detection testing
      - Sterilize sub-set for distribution to community for geochemistry
        - Essential for scientific analysis and assessment of biohazard
        - Radiation sterilization has minimal effects on geochemistry

8



## Control of Back Contamination of Earth (continued)

- Additional SRF considerations
  - Anticipate hazard assessment based primarily on tissue/cell culture
  - Some hazard assessment done at other containment facilities
  - Build SRF from modular, modest-sized, commercially available, biosafety laboratories
  - SRF built, staffed, and operational 1-2 years before sample receipt
- Samples transferred to curation facility after proven safe in SRF

9

**“Current State of Controversy About Traces of Ancient Martian Life in Meteorite ALH84001,” Allan H. Treiman, Lunar and Planetary Institute, Houston, Texas, Feb. 2000.**

Editor's Note: The background tutorial on the status of the scientific debate concerning the ALH84001 meteorite, which was presented at Workshop 1 by A.H. Treiman, was essentially identical to the summary he prepared for the pre-Workshop 1 reading materials. Therefore, to avoid duplication of materials in this report, the outline he submitted for the pre-Workshop 1 reading materials is included in Appendix C (see page 80 in ‘Summaries of Key Planetary Protection Reports’), but is not also included here with the other tutorials.

LESSONS FROM APOLLO*LUNAR SAMPLE QUARANTINE &  
SAMPLE CURATION*

Judith H. Allton  
NASA/Johnson Space Center  
Advanced Curation Team

1

LESSONS FROM APOLLO*Three Responsibilities*

- Fly the missions safely, on schedule
  - Mission managers
- Protect the Earth from biohazard
  - Interagency Committee on Back Contamination
    - NASA, USPHS, Dept. Agriculture, Dept. Interior
- Preserve scientific integrity of samples
  - Lunar Sample Allocation Planning Team
    - a peer review advisory committee

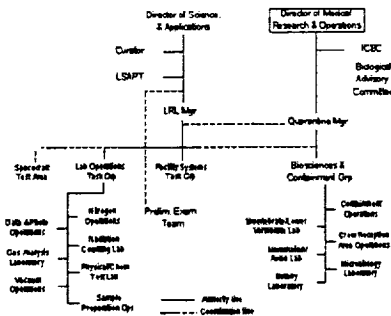
2

LESSONS FROM APOLLO*Lunar Receiving Laboratory*

- Sample receiving, quarantine testing, crew isolation, gas analysis, radiation counting
- 8000 m<sup>2</sup>, \$24M (~\$125M today's dollars)
- 300 persons working 3 shifts
- LRL not adequate for curation, samples moved after Apollo 17 PET complete
  - one concern was organic contamination of sterilant residues, working at negative pressures

3



LESSONS FROM APOLLO*Lunar Receiving Laboratory*

4

LESSONS FROM APOLLO*Baylor Protocol*

- Rationale:
  - "The prime purpose of the laboratory would be to provide a formal mechanism for testing appropriate representative lunar samples for the possible presence of agents that might be infectious or toxic to man, animals, and plants. It should be the goal of this laboratory to provide safety clearance for lunar samples, if possible, within a period of approximately 30 days."
- Three Part:
  - crew microbiology
  - in vitro culturing from lunar material
  - direct challenge with lunar material

5

LESSONS FROM APOLLO*Baylor Protocol*

- Ten phyla comprised of 69 species
  - Priority testing included protozoa, hydra, planaris, nematodes, earthworm, snail, oyster, sea urchin, brine shrimp, cockroach, amphioxus, minnow, salamander, turtle, fowl, mouse.
  - Nine divisions comprised of 43 species for plant challenge
  - Priority included bacterium, green alga, fungus, pine, wheat, bean

6

LESSONS FROM APOLLO*Baylor Protocol*

- Sequence
  - culturing, direct challenge
  - secondary culturing: isolation, identification, animal and plant challenge if suspected hazard
- Exposure techniques
  - ingestion: add to food or water
  - injection: intracerebral, intraperitoneal, intravenous, intranasal, intracutaneous, subcutaneous, oral

7

LESSONS FROM APOLLO*Baylor Protocol*

- Evaluation
  - Determine changes in condition resulting from:
    - natural cause not related to lunar material
    - chemical reaction to lunar material
    - replicating organism from lunar material
  - Look for subtle effects
  - Always use controls

8

LESSONS FROM APOLLO*Baylor Protocol*

- Subtle Effects...
  - oxidative metabolism, reproductive capacity, catabolic activity
  - motility, morphology, size, gas exchange, substrate utilization
  - nucleic acid metabolism, carbohydrate metabolism, lipid metabolism, protein synthesis

9

LESSONS FROM APOLLO*Protocol Implementation*

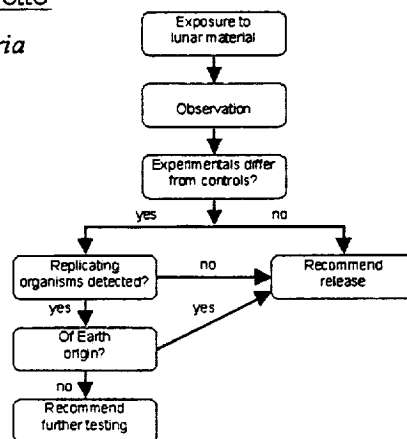
- Objectives:
  - “To determine whether the lunar material presents a significant infectious disease hazard to the terrestrial biosphere. In the event of crew illness it is to determine if the lunar sample contains infectious material in order to establish the etiology of the crew illness.” Goal to certify samples within 30 days using both *in vivo* and *in vitro* challenge systems.
- Class III cabinetry behind secondary barrier

10

LESSONS FROM APOLLO*Protocol Implementation*

- Direct observation, 1000x
- Bacteriology/mycology
- Virology and mycoplasma
- Mammalian
- Invertebrates & fish
- Botany

11

LESSONS FROM APOLLO*Release Criteria*

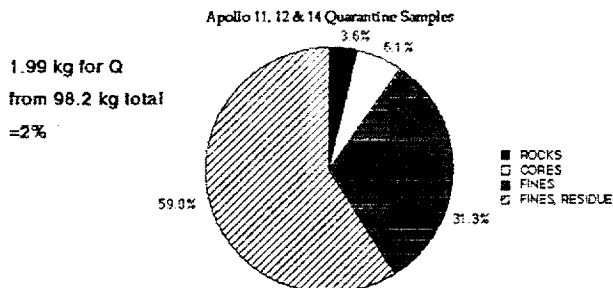
12

## LESSONS FROM APOLLO

## Sample Usage

SCIENCE SAMPLES: Numerous aliquots of small size

HAZARD TESTING: Fewer, larger samples - BIOPOL



13

## LESSONS FROM APOLLO

Discipline	PI	Ave. wt. Soil core	# core samples	Ave. wt. fines	# fines samples	Ave. wt. Rocks	# rocks samples
Isotopes, solid source	Nyquist, Wasserburg	0.1	107	1.38	202	1.94	778
Isotopes, noble gases	Eggert, Geiss, Marti	0.14	234	1.13	205	1.3	422
Isotopes, light elements	Clayton	1.46	115	3.2	71	1.35	158
Cosmogenic nuclides	Arnold	0.23	401	0.28	31	18.56	32
Rock petrology	James, Lofgren, Pyde					0.19	1214
Soil petrology	McGraw, Delano	0.05	810	0.29	528		
Trace elements	Haskin	0.07	1327	0.27	570	0.75	338
Sedimentary v. t. d. l. s.	Gibson	0.11	96	1.97	53	6.34	96
Spectral reflectance	Adams, Pieters	0.1	33	0.64	349	0.68	155
Magnetics	Strangway	0.13	29	2.52	47	21.58	180
Org. Carbon/Micro biology	Burlingame, Oyama	0.30	84	4.26	93	3.81	53
Fossils	Schopf	0.05	3	0.33	32	0.11	26
Quarantine		7.65	18	15.35 (non-core)	48 (non-core)		

14

## LESSONS FROM APOLLO

## Need to minimize organics

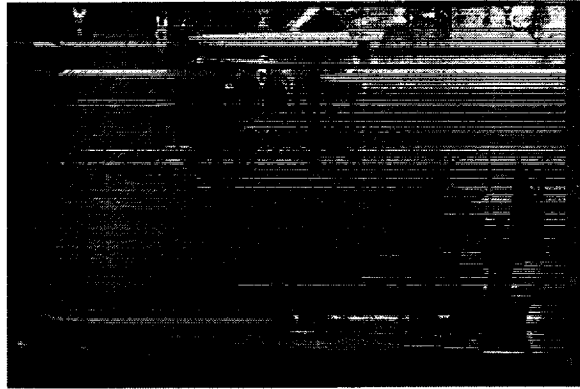
Southwest Research Biomedical  
BSL-4PVC suits, organic sterilants in shower &  
dunk tank

15

LESSONS FROM APOLLO*Need to Minimize Organics*

Dugway Half-suit Glovebox

PVC suit, plastics



16

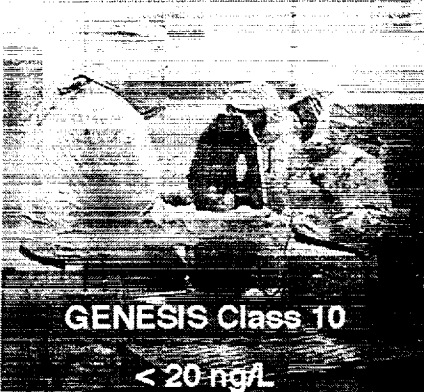
LESSONS FROM APOLLO*Making a Clean Lab  
Cleaner*

- Typical cleanroom culprits....
  - ULPA, HEPA media & sealants
  - plasticizers from flooring and wall covering
  - adhesives in tape, piping, walls & flooring
  - plastic equipment & bags
  - glovebox gloves & heat sealers
- Minimization through material control & sorption

17

LESSONS FROM APOLLO

*Making a Clean Lab Cleaner*



**GENESIS Class 10**  
**< 20 ng/L**

**N<sub>2</sub> glovebox**  
**<10 ng/L**

N,N-dibutyramide  
N,N-dibutyramid  
Diethyl phthalate  
Caprolactam

Dimethylcyclohexanol  
Diisopropylbenzene  
Isopropylacetophenone  
Alkyl ester  
Diethylbenzene  
Diisobutylphthalate  
Dibutylphthalate  
various stoxanes

18

LESSONS FROM APOLLO

*Conclusions*

- Combining CLEAN, STERILE and CONTAINED is difficult technically
- Minimum sample to be used for hazard testing
- Minimum sample handling reduces contamination

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# **Report on Mars Sample Quarantine Protocol Workshop**

**D.L. DeVincenzi, J. Bagby, M. Race, & J.D. Rummel  
(eds.)  
NASA CP-1999-208772  
NASA Ames Research Center  
Moffett Field, California, (1999).**

1

## **Background**

- **1996: MELTSWG determined need for study of five specific PP areas**
- **One of the priority needs: To develop guidelines for containment and quarantine analysis of returned martian samples.**
- **Convened Mars Sample Quarantine Protocol Workshop-- June, 1997**
- **Update containment procedures and testing methodology since Apollo**

2

## Workshop focused on three aspects of handling returned Mars sample:

- **Biocontainment**-- to prevent uncontrolled release of sample material into the terrestrial environment
- **Life detection** -- to examine the sample for evidence of live organisms or biological remnants
- **Biohazard Testing**-- to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere.

3

## Basic Assumptions

- Sample Return mission launched in 2005
- Samples returned from biologically interesting sites based on data from '96, '98, '01, & '03 missions
- Sample will not be sterilized prior to return to Earth
- Amount of sample for quarantine tests will be a small fraction of the total amount returned
- Biocontainment of the unsterilized sample maintained until quarantine testing for biohazards is accomplished .

4



# 1. Containment Sub-Group

- **Recommendations for safe, controlled management of Mars sample**
- **Containment = System for**
  - Protection of Earth's biosphere from release of 'biological entities' of martian origin
  - Maintain the integrity of the sample
- **Recommendations for**
  - Sample Return Canister
  - Containment Needs and Procedures en route
  - Mars Receiving Lab Containment (including alternative approaches)

5

## Containment Sub-Group : Sample Return Canister

Entire containment system– from Mars to Earth –  
must prevent the escape of potentially hazardous material

- **Decontamination of canister exterior that contacts martian surface**
- **Contingencies for non-nominal events during transit to Earth**
  - e.g. Bias trajectory of ERV to miss Earth;
  - Indicator system to monitor for breach of containment en route;
  - On board system for sterilization in case of an in flight containment breach;
  - Provisions to determine if a breach occurs during hard landing ,
  - Suitable sterilization for landing breach
- **Reconfirmation of proper containment upon canister recovery,**
- **Canister transported to quarantine facility in suitable container**
  - Meet regulatory requirements for transport of potentially hazardous biological material
- **Provisions for protective garments for the recovery crew**
- **Coordination with appropriate regulatory agencies**

6

### Containment Sub-Group : Mars Receiving Lab (MRL)

Unknown nature of possible biohazardous material in sample  
 requires use of most stringent biocontainment-- BSL-4 operation  
 (Alternative approaches possible- TBD)

- **Primary Containment via Class IIIBiosafety Cabinets (BSC)**

- Glove boxes connected in sequence with sealable doors between cabinets and maintained under negative pressure

- **Secondary Containment provided by building**

- High-end BSL-3 structure--sealed and maintained under negative pressure
- HEPA filtered exhaust air, sterilized waste water, provision for personnel showers & use of disinfectants

- **Glove Box System: Flexibly designed to include lab equipment for protocols**

- Operational parts of equipment inside-- electronics/ controls outside

- **Careful planning for sequence of handling, opening etc. to avoid contamination during containment**

7

### Containment Sub-Group :

### Research and Technology Needs

- **Challenge tests of HEPA filtration system** using carbon-bearing particles 10 nm to 100 nm in size
- **Choose appropriate isotopes and particle sizes** for use in flight verification and testing of canister seals (e.g., carbon compounds, radioactive-tagged particles)
- **Select appropriate indicator for canister seal integrity** upon recovery
- **Design processes to clean containment area** of terrestrial biological entities and organics to avoid false positives
- **Develop and test systems to maintain sample integrity** when obtaining aliquots of material for quarantine testing
- **Design a system for needle puncture of the 'head space'** through a vacuum-sealed line
- **Determine suitable sterilization methods** for Mars sample

8

## 2. Life Detection Sub-Group

- **Develop a series of tests (protocol) to detect presence of**
  - **Live organisms or**
  - **Materials derived from live organisms**
- **Need to Detect Life *and* Distinguish Terrestrial Contamination**
- **Important Factors:**
  - **Sequence of Tests**
  - **Multiple Lines of Evidence for martian origin**
  - **Essential to understand geological and ecological context**
  - **Strong Quality Control Program including use of tracers correlated with different mission phases**

9

### Life Detection Sub-Group:

#### **Need to Establish Context for Life Detection in Sample**

- **Preliminary Analyses recommended**  
(Assumes tests done both in- and outside quarantine)
  - **Characterize bulk mineralogy of the sample**
  - **Establish elemental composition**
  - **Inventory volatile and organic materials**
  - **Measure redox couples present in sample material**
  - **Obtain microscopic characterization of sample surface and interior**

10

## Life Detection Sub-Group: Recommendations

- **Three Basic Methods for Life Detection**

1. **Organic Chemical Analysis and Detection**

(search for functional groups w/ C,S, N; detection of AA's , proteins, fatty acids, carbohydrates, nucleic acid bases, etc; cell wall components; etc)

2. **Light and/or Electron Microscopy**

(morphological indications of life; staining; w/ trace mineralogy)

3. **Cultivation Techniques -- Multiple Media & Conditions**

(segue to hazard detection; potential to amplify 'signal' of living entities; acknowledge low culturability of Earth microbes)

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## Life Detection Sub-Group: Research and Technology Needs

- **Incorporate life detection technologies into planning and sample receiving activities for MSR mission**
- **Develop Plan for acquisition and operation of appropriate instrumentation within the sample handling facility**
- **Develop appropriate sterilization protocols that minimally impact science return**
- **Develop methods to prepare samples for distribution to the wider scientific community**

12

### 3. Biohazard Sub-Group

- **Develop up-to-date methodology to determine if returned sample materials are hazardous, regardless of whether life or biological entities are detected**
- **Tiered or stepwise approach**
  - Use protocols that screen for wide range of biological agents
  - Look for indications of biological activity or disruption thereof,
  - Incorporate systematic feedback from life detection studies , chemical analyses, and biohazard tests themselves
- **Emphasis on hazards posed by organisms that replicate**
- **Focus on two priority biohazard concerns**
  - **Pathogenicity** (infectious and bio-toxins) & **Ecological Disruption**
- ***In vitro* tests superior to whole organism studies**

13

#### Biohazard Sub-Group: Recommended Battery of Tests

- **Bacteriological Media**-varied conditions and media
- **Selected Tissue Cultures/ Cell Lines**-mammalian organ systems, fish, insects
- **Embryonated Chicken Eggs**
- **Mouse Injection Studies**
- **Plant Tissue Culture** (Tobacco, Wheat, Rice, Potato)
- **Tetrahymena (Protozoans)**
- **Microbial Microcosms** (ecosystem effects; disruption of biogeochemical cycles)

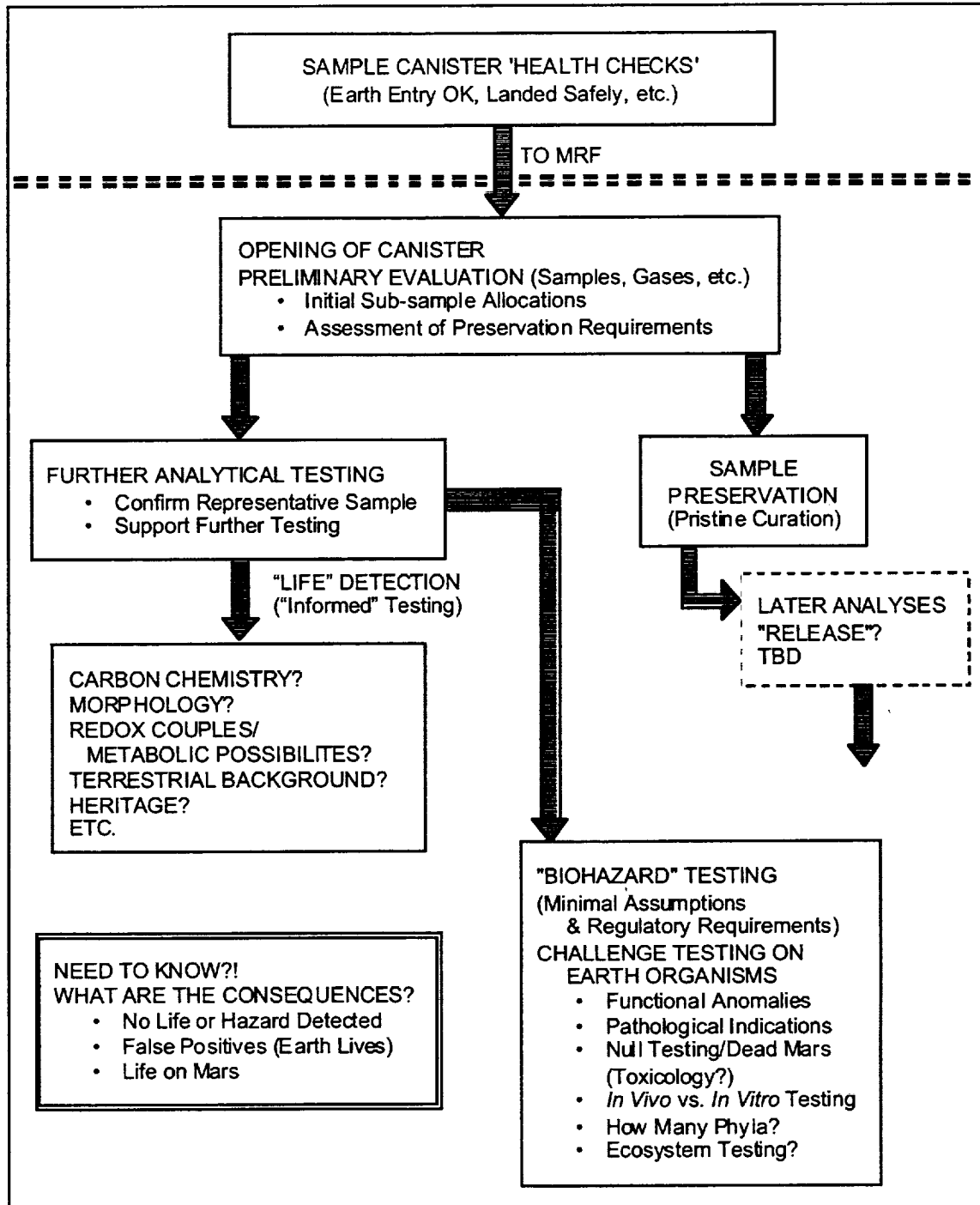
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## Biohazard Sub-Group: Research and Technology Needs

- **Validation of Methodological Approach** (cell and tissue tests rather than whole organisms studies; pre-testing of efficacy; etc.)
- **Microcosm Research** (development, effectiveness; predictive value; non-destructive, long-term observation and sampling, etc.)
- **Representative Samples, Controls and Replicates**
- **Other Operational Issues** (training and monitoring programs for lab personnel; management of lab operations and facilities; issues related to limited quantities of material, sample allocation, research access, and evaluation of research proposals).

15

**DRAFT OVERVIEW: MARS SAMPLE RETURN PROTOCOL**  
**A Working Guideline for March 2000 Workshop 1 Deliberations**  
 (Presented by J. Rummel)







## **APPENDIX E:**

## **REFERENCES**

- Carr, Michael H., et. al. (1999), "Mars Sample Handling and Requirements Panel (MSHARP) Final Report," NASA, Jet Propulsion Lab, Pasadena, California, April 1999, NASA TM-1999-209145.
- DeVincenzi, D.L., J. Bagby, M. Race, and J.D. Rummel (1999), "Mars Sample Quarantine Protocol Workshop," NASA Ames Research Center, Moffett Field, California, June 1997, NASA CP-1999-208772.
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- Space Studies Board (1992), "Biological Contamination of Mars: Issues and Recommendations," Task Group on Planetary Protection, chaired by Kenneth Nealson, National Research Council, National Academy Press, Washington D.C.
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## APPENDIX F: GLOSSARY

BSL	Biosafety Level
CDC	Center for Disease Control (US)
CNES	Centre National d'Etudes Spatiale (French)
CNRS	Centre National de la Recherche Scientifique (French)
COMPLEX	Curation and Analysis Planning Team for Extraterrestrial Materials
EPA	Environmental Protection Agency (US)
GERT	Graphical Evaluation and Review Technique
HEPA	High Efficiency Particulate Air (filter)
IDP	Interplanetary Dust Particle
IR	Infrared
IUS	Inertial Upper Stage Engine
LAL	Limulus Amebocyte Lysate
MCL	Maximum Containment Laboratory
MELTSWG	Mars Exploration Long Term Science Working Group (US)
MRL	Mars Receiving Laboratory
MS	Mass Spectroscopy
MSHARP	Mars Sample Handling and Requirements Panel (US)
MSR	Mars Sample Return
MSRV	Mars Sample Return Vehicle
NASA	National Aeronautics and Space Administration (US)
NASA-SP	NASA Special Publication
NASA-TM	NASA Technical Memorandum
NRC	National Research Council (US)
OQF	Orbiting Quarantine Facility
PCR	Polymerase Chain Reaction
PP	Planetary Protection
TELLE	Remote-Teleoperated-Manipulator System
SPF	Specific Pathogen Free
SRC	Sample Return Canister
SRF	Sample Receiving Facility
SSB	Space Studies Board
TBD	To Be Determined
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USDA-APHIS	U.S. Department of Agriculture, Animal and Plant Health Inspection Service
UV	Ultraviolet



## APPENDIX G: TEXT NOTES

- Note 1. The summary reports presented in this document (including tables and figures) reflect the deliberations of each Sub-group. The findings are preliminary and there may be inconsistencies between the Sub-groups. The views expressed and any conclusions and recommendations reached by the Sub-groups do not represent a consensus of all Workshop participants and may not necessarily be consistent with the final report and recommendations to be issued at the conclusion of the Workshop series.
- Note 2. Although there were six assigned topics and six Sub-groups, there are only five Sub-group summaries in this report. The chairpersons of Sub-groups 2 and 4 elected, because of the large degree of overlap in the discussion topics between their two Sub-groups, to write a single, combined summary report to cover their two Sub-groups' discussions.
- Note 3. According to the 1997 SSB report on Mars Sample Return (p. 29), "... if any portion of the sample is removed (from containment) prior to completion of analyses, it should first be sterilized." (p. 4). Moreover, "... if viable exogenous biological entities are discovered in the sample material, prudence would indicate that they remain segregated from the Earth's biosphere (i.e., they should remain in containment or be made non-viable through sterilization) ..." and "... if viable biological entities are discovered in sample materials returned from Mars, and those entities cannot be accounted for by terrestrial organisms which had been conveyed on the outbound spacecraft, then the sample material should be deemed hazardous and no portion should be removed from containment without first being sterilized."
- Note 4. To date, no decisions have been made about when and under what conditions sample materials will be eligible for or will actually be released from containment at the SRF. Even if no biological entities are detected, samples may still be subjected to rigorous biohazard testing before decisions about release from containment can be made. Questions regarding release of materials will be discussed in later Workshops, and will invariably involve considerations about sample sterilization and interpretation of protocol test results. Ultimately, it is likely that decisions about what is done with sample materials will be made after review by an appropriate international scientific oversight committee at the SRF in consultation with NASA's Planetary Protection Officer and other responsible officials.
- Note 5. To date, no decisions have been made about sterilization of sub-samples, including the method(s) to be used. Sterilization questions and issues will be addressed in detail in a subsequent NASA planetary protection Workshop on sterilization methodology.
- Note 6. No decisions have been made on the amount of sample material that will be used for preliminary testing, life detection tests, or biohazard analysis. Some destructive testing of sample materials will probably be necessary in the course of implementing the actual protocol.
- Note 7. No decision has been made to date on whether a single or multiple facilities might be utilized to carry out the sample handling protocols. It is possible that specialized testing equipment or infrastructure at locations separate from the SRF may be used as part of the sample handling protocol, with the presumption that appropriate containment and transportation methods would be used if and when samples are moved between facilities.

- Note 8. For the purposes of this protocol, biological entities of concern would include those that are either active or dormant. Fossilized entities would be of great interest, but would not necessarily dictate continued containment because they are incapable of replication.
- Note 9. Sub-group 5 proposed a number of life detection methods that may have ultimate fundamental applications to a Mars sample return protocol. However, the Sub-group's proposal was necessarily cursory in light of the time allowed for discussion. The proposed methods were not refined to address the unique characteristics of anticipated martian samples, and efforts to detect or preclude terrestrial contaminants were only minimally identified and discussed. These limitations will be addressed and reconciled in subsequent Workshops that focus on both life detection and sterilization.



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