NASA/TM-1999 – 209145

Mars Sample Handling and Requirements Panel (MSHARP) Final Report

Michael H. Carr	*U.S. Geological Survey, Menlo Park, CA*
Daniel J. McCleese	*Jet Propulsion Laboratory, Pasadena, CA*
Jeffrey L. Bada	*Scripps Institution of Oceanography, La Jolla, CA*
Donald D. Bogard	*Johnson Space Center, Houston, TX*
Benton C. Clark	*Lockheed Martin Astronautics, Denver, CO*
Donald DeVincenzi	*Ames Research Center, Moffet Field, CA*
Michael J. Drake	*University of Arizona, Tucson, AZ*
Kenneth H. Nealson	*Jet Propulsion Laboratory, Pasadena, CA*
James J. Papike	*University of New Mexico, Albuquerque, NM*
Margaret S. Race	*SETI Institute, Mountain View, CA*
David Stahl	*Northwestern University, Evanston, IL*

National Aeronautics and Space Administration

Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

April 1999

This publication was prepared by the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. It contains results of research and planning performed by a number of government and contractor facilities performed with funding provided by the National Aeronautics and Space Administration Headquarters. The institutions involved are listed in the introduction.

Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement by the United States Government or the Jet Propulsion Laboratory, California Institute of Technology.

ABSTRACT

In anticipation of the return of samples from Mars toward the end of the first decade of the next century, 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. 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.

TABLE OF CONTENTS

1 INTRODUCTION 1
1.1 Charter of the Mars Sample Handling and Requirements Panel 1
1.2 Background 2
1.2.1 Mars Exploration Goals 2
1.2.2 Sample-Return 3
1.3 NRC Findings and Recommendations 4
1.3.1 NRC Findings 6
1.3.2 NRC Recommendations 6
2 OVERVIEW 8
2.1 Sample Preservation and Contamination Control 8
2.2 Prevention of Uncontrolled Introduction of Martian Materials Into the Terrestrial Environment 9
2.3 Hazard Assessment 9
2.4 Maximize Science Return 9
2.5 Alternative Approaches Considered and Rejected 10
2.5.1 Return Samples to Space Station 10
2.5.2 Return Only Sterilized Samples 11
2.6 Risks of Returning Unsterilized Samples 13
3 SAMPLE ACQUISITION 15
3.1 Overview 15
3.2 Sample Types 17
3.2.1 Rock Cores 17
3.2.2 Contingency Sample 17
3.2.3 Soil Samples 18
3.2.4 Atmosphere Sample 18
3.3 Sampling Strategy 18
3.3.1 Sample Separation 19
4 PREVENTION OF SAMPLE CONTAMINATION 20
4.1 Overview 20
4.2 Approach 22
4.3 Sterilization and Cleaning 22
4.3.1 Sterilized and Superclean Components 22
4.3.2 Other Components That Land on Mars 23
4.3.3 Earth Return Vehicle 23
4.4 Contamination Monitoring 23

4.4.1 Tracers 23
4.4.2 Witness Plates 24
4.4.3 Assays 24
5 TRANSPORT OF SAMPLES FROM MARS TO EARTH 25
5.1 General Statement 25
5.2 Sample Containment 25
5.2.1 Nominal Earth Entry Conditions 25
5.2.2 Anomalous Earth Entry Conditions 27
5.3 Contingency Sterilization 27
5.4 Sample Preservation 27
5.5 Aseptic Transfer 28
6 SAMPLE HANDLING ON THE GROUND 29
6.1 General Approach 29
6.2 Apollo Experience 33
6.3 Sample-Handling Facilities 33
6.4 Sample Receiving Facility 34
6.4.1 SRF Functions and Their Implementation 34
6.4.2 Options to be Considered in Design of Sample Receiving Facility 39
6.5 Sample Curation Facility (SCF) 40
6.6 Management Issues 41
6.6.1 Planetary Protection Management 41
6.6.2 Science Management 41
6.6.3 PET 42
6.6.4 CAPTEM 42
7 SUMMARY AND CONCLUSIONS 43
8 REFERENCES 46

APPENDICES

A Effects of Dry Heat Treatment on Bacterial Survival (Implications for Mars Sample Return) A-1
B CAPTEM Inputs on Sample Return B-1
C Acronyms and Unusual Terms C-1

LIST OF FIGURES

Figure 3-1 Mars Sample Return Missions: 2003, 2005 Opportunities 16
Figure 4-1 Mars Sample-Return Contaimination Control 21
Figure 5-1 Earth Entry Capsule (March design from M. Adler, JPL) 26
Figure 6-1 Delivery of the Samples to the Containment Facility 30
Figure 6-2 Early Processing of Samples at the Containment Facility 31
Figure 6-3 Later Sample Processing at the Containment Facility 32

LIST OF TABLES

Table 5-1 Conditions Recommended for Preservation of Martian Samples by Gooding (1990) 28
Table 6-1 Example of a Mars Sample Processing Plan 40

1 INTRODUCTION

1.1 Charter of the Mars Sample Handling and Requirements Panel

In anticipation of the return of samples from Mars nearly a decade into the next century, NASA in 1996 requested, that the National Research Council (NRC) advise the agency on planetary protection issues connected with the return of samples from Mars to the Earth. The NRC appointed a Task Group on Issues in Sample-return (TGISR), and its findings were published early in 1997 (National Research Council, 1997). In November 1997 NASA established the Mars Sample Handling and Requirements Panel (MSHARP) to advise how the recommendations of the NRC panel might be implemented. The panel was convened by the Office of Space Sciences at NASA Headquarters, and it was to report to the Associate Administrator for Space Sciences. It was chartered to recommend requirements in three areas:

1. Sample collection and transport back to Earth
2. Certification of the samples as non-hazardous
3. Sample receiving, curation, and distribution.

The NRC reports on Biological Contamination of Mars and Mars Sample-return (National Research Council, 1992, 1997) were to serve as guides in making the panel's recommendations. MSHARP's concern is with sample-return. It is concerned with forward contamination of Mars only insofar as it affects sample return.

The membership of MSHARP changed during the panel lifetime but included the following:

Michael H. Carr, Chair, U. S. Geological Survey, Menlo Park, CA

Jeffrey L. Bada, Scripps Institution of Oceanography, La Jolla, CA

Donald D. Bogard, Johnson Space Center, Houston, TX

Benton C. Clark, Lockheed Martin Astronautics, Denver, CO

Michael J. Drake, University of Arizona, Tucson, AZ

Donald DeVincenzi, Ames Research Center, Moffet Field, CA

Daniel J. McCleese, Jet Propulsion Lab., Pasadena, CA

Kenneth H. Nealson, Jet Propulsion Lab, Pasadena, CA

James J. Papike, University of New Mexico, Albuquerque, NM

Margaret S. Race, SETI Institute, Mountain View, CA

David Stahl, Northwestern University, Evanston, IL

1.2 Background

1.2.1 Mars Exploration Goals

The focus of the Mars exploration program is the comprehensive goal of understanding the suitability of Mars as a possible abode of past or present life. We wish to understand Mars well enough to determine if some form of life ever evolved there, and if not, why not. Present conditions at the martian surface are very hostile, but geologic evidence suggests that the planet was more hospitable in the past. The initial emphasis of the program is, therefore, to look for evidence of past life. Essential elements in understanding Mars as a possible abode for life are determining the planet's climate and geologic histories and achieving a better understanding of why they have been so different from the Earth's. These are, therefore, parallel goals to the direct search for past and present life.

The martian surface is expected to be sterile because of the hostile conditions (see summary of Mars surface conditions in Kaplan, 1988; Carr, 1996). These are such that liquid water, which is universally held as essential for life, is unstable everywhere. The partial pressure of water is approximately one microbar at the surface. Surface temperatures at the equator typically range from –100°C just before dawn to peaks during southern summer at 0° to 20°C, depending on the properties of the surface materials. Under these conditions, not only is liquid water unstable at the surface, but at low latitudes ice is unstable. It will tend to sublime into the atmosphere, and the water vapor will ultimately be precipitated out at the poles. In addition to the lack of liquid water, 0.2–0.3 μm (2000–3000 Å) ultraviolet (UV) radiation from the Sun passes unattenuated through the martian atmosphere (Kuhn and Atreya, 1979) and appears to cause breakdown of any organic molecules that may be at the surface. The surface material is also oxidizing, thereby contributing further to the breakdown of organics. As a result, the Viking landers found no complex organic molecules at the parts per billion (ppb) level despite expectations of their presence at this level simply from meteorite infall (Biemann et al., 1977). The martian surface and the dust in the atmosphere are, thus, both expected to be sterile. If there is any life near the surface, it would have to be within rocks or below the surface where there is protection from the UV radiation, and it would also have to have the capability of sequestering water in the physically unfavorable environment

Optimism that some form of life could have started on Mars arises not because of present conditions on the planet, but because of indications in the geologic record that conditions were different in the distant past. Large channels, seemingly formed by gigantic floods, indicate that large quantities of water episodically flowed across the surface at times in the past. Also the presence of numerous smaller dry valleys supports the supposition that, in the very distant past, climatic conditions were such that liquid water could have been stable at the surface (summarized in Carr, 1996). The time that most of these valleys formed is prior to 3.5 billion years ago. We know that by this time life had started on Earth. We do not know what happened on Mars, but both planets at this time had conditions that would seem favorable to life: water-rich; high rates of volcanism; high rates of impact; and seemingly warmer surface temperatures that allowed liquid water at the surface. The initial exploration strategy is, therefore, to acquire samples of rocks that formed early in Mars' history and that record the conditions at that time.

It has been argued that if some form of life did start on early Mars, when conditions were more hospitable, then as Mars evolved to the hostile planet we know today, the organisms could have adapted to survive in niches where liquid water was available and where they were protected from the UV radiation and oxidizing conditions at the surface. Examples

of such possible niches are aquifers that may exist kilometers deep below the surface or active volcanic systems, although these have yet to be detected.

Although detection of past life is a primary goal of the exploration program, it is not the only goal. Searching for evidence for past or present life on Mars, while enormously important, is a high-risk venture, scientifically. Life may not have developed on Mars, and even if it did, we may not be able to immediately find evidence for it. On the other hand, any samples returned from Mars will yield evidence of the climatic and geologic evolution of the planet. Moreover, there may be evidence of pre-biotic chemistry that under more favorable circumstances could have led to the origin of life.

Determination of the climatic history of Mars is of considerable interest, not only for assessing the prospects for past life and the conditions under which life might arise, but also for understanding the early history of the Earth and why the climatic evolution of the two planets was so different. The geologic histories of Mars and Earth have also been very different, and samples will enable us to understand why. These climatic and geologic goals are of low risk scientifically in that returned samples will inevitably lead to their better understanding.

A final practical goal for Mars exploration is to prepare the way for human exploration. Of special interest is determining whether the omnipresent dust is harmful to people or machines.

1.2.2 Sample-Return

Sample-return has long been a goal of Mars Exploration, and many of the outstanding biologic, geologic, and climactic issues with respect to Mars are unlikely to be resolved until we have a variety of returned samples. We already have, of course, some samples that have been naturally returned to Earth – the martian meteorites, of which we now have thirteen. These meteorites are all igneous rocks, and they have already yielded fundamental information about Mars, e.g., that the planet underwent global differentiation at the end of accretion, and some of the near-surface rocks have suffered alteration by groundwater. The meteorites have also provided tantalizing suggestions that life could have started on the planet as early as 3.9 billion years ago (McKay et al., 1996). Our goal with sample-return missions is not only to supplement our suite of igneous rocks, but to acquire samples of materials such as soils, sediments, and hydrothermally altered rocks, which are more likely to yield information on climate, biology, and geology than the igneous rocks alone, and which are not represented among the martian meteorites.

The advantages of having samples available for analysis here on Earth, as opposed to being dependent on in-situ analyses, are enormous (Gooding et al., 1989; Jones and Treiman, 1998). The accuracy and precision possible in terrestrial labs is usually orders of magnitude better than is possible with in-situ analyses. Determination of oxygen isotopes, for example, is a thousand times more precise in terrestrial labs than has so far been possible at Mars. The oxygen isotopes determined in martian meteorites show that there is water from at least two sources in the meteorites. This water includes juvenile water with the same isotopic composition as the rocks and water that have been in the atmosphere and have been exposed to fractionating processes. This conclusion, which is important for understanding how the atmosphere has evolved, could not have been reached with the low precisions of in-situ analyses.

In addition, many techniques, such as those required for age dating, are so complex that they strain the capabilities of terrestrial laboratories; they are simply too complex to be packaged and sent to another planet. Other instruments may be relatively simple, but sample

preparation is difficult, as for example preparation of thin sections for optical microscopy or making thin/film casts for transmission electron microscopy.

Other disadvantages of in-situ analyses are that they are necessarily restricted to small predetermined sets and that the instruments tend to be chosen, not necessarily by the importance of the data they produce, but by our ability to compress the instruments into small masses and volumes for spaceflight and by their ability to accept simply prepared samples. Thus, the alpha proton x-ray spectrometer (APXS) instrument, which determines major element composition, is planned to be flown on several Mars lander missions because it is small, simple, and requires no sample preparation. However, despite the widely acknowledged desirability of getting age dates on martian rocks, there are no plans to send instruments to do age dating. The instruments required are too complex, and the sample preparation is too elaborate. The choice of what information to be obtained is thus partly controlled by instrument complexity, not by the desirability of the data.

With samples here on Earth, the entire analytical capability of the science community can be used, and hundreds of sophisticated analytical techniques can be applied to the samples. Moreover, the analytical strategy can shift in response to what is found in the samples, or new techniques can be developed, if needed. Many of the techniques currently being used to analyze lunar samples, for example, were not in existence when the samples were initially returned. Finally, work can continue on the samples as ideas mature and new techniques become available. Lunar samples are still undergoing analyses and yielding new results 30 years after they were collected.

The experience with the martian meteorite ALH84001 is instructive. More than 100 metric tons of instruments and laboratory equipment have been used in analyzing ALH84001. In contrast, we can typically expect no more than a few tens of kilograms for instruments on a lander mission to Mars.

1.3 NRC Findings and Recommendations

Given the strong desire for sample-return, the NRC Task Group on Issues in Sample Return (TGISR) (National Research Council, 1997) was asked by NASA to assess the potential of the samples for negative impacts on the Earth's biosphere and to make recommendations as to what steps should be taken to mitigate those possible effects. The task group concluded that the potential for large-scale effects from martian samples, either through pathogenesis or ecological disruption, is extremely small, although it cannot be demonstrated to be zero. Several observations support this conclusion. First, the chances of any viable organisms being in the sample are low. As already indicated, the lack of liquid water, the radiation environment, and the presence of oxidants in the soil render the surface very hostile. It is conceivable that some form of microbial life could have started on early Mars, or been introduced from Earth by meteorites, and survived to the present day in protected niches well below the surface. Impacts could have brought the putative microbes to the surface within rocks. But most rocks at the martian surface are likely to be hundreds of millions of years old, and overcoming the radiation damage accumulated over such long periods is unlikely. Microbes could also be introduced onto the surface through volcanic vents and incorporated into the soil, but survival again is extremely unlikely in view of the radiation, the oxidants, and the likely long residence times in the soil.

Second, even if there were viable organisms in the samples, the potential for pathogenic effects is small. Pathogenesis can be divided into two fundamental types: toxic and infectious, both of which are potential concerns in screening returned samples. Generally, toxic effects of microorganisms are attributable to cell components or metabolic

products that incidentally damage other organisms. Infectious agents have the capacity to multiply in or on the host and cause damage. The capacity of a microbe to infect a host usually involves an intimate interaction between the pathogen and the host. Infection often depends on highly specific interactions between cell surfaces of the host and pathogen, and it must overcome the defenses that have evolved in potential hosts. The chances that invasive properties would have evolved in putative martian microbes in the absence of evolutionary selection pressure are small. However, as noted in a subsequent NRC report on sample-return from solar system bodies (NRC, 1998), because there are examples of opportunistic pathogens from terrestrial and aquatic environments that have not co-evolved with their host, the risk is not zero.

Third, the NRC study on Mars sample-return (1996) noted that it is unlikely that putative martian organism would cause any widespread ecological disruption. They would likely be functionally similar to some terrestrial organisms and utilize nutrients that terrestrial organisms already consume efficiently. Moreover, it is unlikely that the martian organism could out-compete Earth's organisms for nutrients since Earth's microorganisms are optimally adapted to the environments as a result of millions of years of intense competition. Laboratory microbes that have been engineered to utilize particular substances at an accelerated rate usually fail in the field because they cannot compete with well-adapted microorganisms that already exist there (Fry and Day, 1992). However, as noted in the NRC small bodies sample-return report (1998), currently there is very little information on the effects of introduced microorganisms on established microbial communities or on spatial or temporal variations in natural microbial environments on Earth.

Fourth, martian meteorites continually bombard the Earth, and they have caused no known adverse effects. Approximately 30,000 meteorites larger than 100 grams reach the Earth every year (Gladman, 1997; Halliday et al., 1989). Among all known meteorites, the ratio of martian to nonmartian is 5 x 10–4. However, martian meteorites are more difficult to recognize than most other meteorites. A better indication of the true ratio may be the ratio among falls, which is approximately 10–2. If these two figures are taken as limits, then 15 to 300 martian meteorites hit the Earth every year. Although most martian meteorites spend millions of years in space where they could be sterilized, a small fraction reach Earth within thousands of years or even a few years (Gladman et al., 1997) and would not be sterilized. Since many are only minimally shocked on leaving the Mars surface, and only the outer few millimeters are heated on Earth entry, it appears likely that the Earth commonly receives unsterilized Mars samples. On the other hand the meteorites may not be representative of the materials available to be sampled at the martian surface. There are, for example, no soils or sediments among the martian meteorites.

For these reasons, the NRC Task Group (NRC, 1997) came to the following conclusion, with which we concur:

> The possibility of life on Mars cannot be excluded on the basis of our understanding of the martian environment. Nevertheless, the potential for including a living entity in a sample-returned from Mars is judged to be low, especially if the sample is returned from a site that has not been specifically targeted as a possible oasis. The potential for returning an organism that could grow and multiply in the terrestrial environment is lower still. If an organism were returned that could survive on Earth, the potential for large-scale ecological or pathogenic effects still would be low. Any organism that could survive in Earth's environment would meet intense competition from well-adapted terrestrial organisms that occupy their habitats to the limits of available resources. It is especially unlikely that putative martian organisms could be agents of infectious disease. Such a capability requires specific

adaptations, for which there is no selection pressure on Mars, to overcome the elaborate defenses against invasion possessed by terrestrial organisms. There are large uncertainties associated with these assessments, however, and the risk of potentially harmful effects is not zero.

In light of the above reasoning, the NRC Task Group made the following findings and recommendations.

1.3.1 NRC Findings

1.3.1.1 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.

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

1.3.1.3 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.

1.3.2 NRC Recommendations

1.3.2.1 Samples returned from Mars by spacecraft should be contained and treated as potentially hazardous unless proven otherwise. No uncontained materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.

1.3.2.2 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.

1.3.2.3 Integrity of containment should be maintained through re-entry of the spacecraft and transfer of the sample to an appropriate receiving facility.

1.3.2.4 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.

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

1.3.2.6 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 2 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 and given oversight responsibility for the facility.

1.3.2.7 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 1 year prior to the establishment of the sample receiving facility (at least 3 years prior to launch)

1.3.2.8 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, re-entry, and sample distribution.

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

2 OVERVIEW

The Sample-return System can be viewed as all the elements involved in acquiring samples, delivering them to Earth, and making them available to the science community for analysis. The system has 3 primary goals:

1. To return martian samples to Earth as unaltered and free of terrestrial contamination as is possible

2. To prevent uncontrolled introduction of martian materials into the terrestrial environment, whether in the sample capsule or on other spacecraft components.

3. To maximize science return from the samples.

Requirements for handling Mars samples are driven by two distinctly different and important emphases: on the one hand, traditional biosafety and planetary protection concerns; and on the other, sample protection and science considerations. The former focuses on keeping materials in, while the latter strives to keep contaminants out. Developing specific containment guidelines for handling extraterrestrial samples will require accommodation of these two needs. It will be a major task to translate established Center for Disease Control National Institutes of Health (CDC-NIH) biocontainment guidelines for known biological agents into laboratory designs, equipment designs, and handling protocols appropriate for operations both in space and on Earth. In addition, the needs of planetary scientists to minimize terrestrial contamination and maintain samples in a pristine and unaltered state down to the isotopic composition level will be added. The end-to-end design of the system for handling the samples will initially need to be under strict containment consistent with biosafety level 4 (BSL-4) as defined by CDC-NIH biocontainment guidelines. At the same time it must incorporate proven techniques for meeting cleanliness criteria of the existing extraterrestrial materials facility at Johnson Space Center (JSC, Houston, TX). In short, it must be compatible with the best available practices for meeting both biosafety and planetary science needs. The steps to be taken to achieve these challenging goals are summarized below.

2.1 Sample Preservation and Contamination Control

Terrestrial contamination of the samples should be minimized by application of appropriate cleanliness standards to different spacecraft components, according to how likely contaminants could be transferred to the samples. Different standards pertain to different potential contaminants such as live cells, dead cells, biogenic molecules, and carbon compounds. All components that come in direct contact with the samples should be both sterilized and cleaned to significantly higher levels than Viking (see Section 4). Since perfect cleanliness cannot be achieved, assays should be made of various potential contaminants, and markers should be used where appropriate (e.g., in lubricants). However such markers should only be used to follow adventitious contaminants and should not be deliberately introduced into the samples, such as by adding tracers to drill bits. To avoid alteration of the sample en route to the Earth, samples should be stored under pressure and temperature conditions similar to those at the surface of Mars, and should not be exposed to vibrations that would destroy the physical integrity of the samples. **To prevent terrestrial contamination the sample capsule should be opened in a facility that matches or exceeds the cleanliness criteria of the existing extraterrestrial materials facility at JSC. This facility must also provide the equivalent containment of a BSL-4 facility, as discussed below. As far as we know, such a facility does not yet exist.**

2.2 Prevention of Uncontrolled Introduction of Martian Materials Into the Terrestrial Environment

The samples must be returned to the Earth in such a way that minimizes the probability of inadvertent introduction of unsterilized martian materials into the Earth's biosphere. This is to be accomplished by enclosing the samples within a canister that is sealed before leaving the martian surface and which is surrounded by additional seals and High Efficiency Particulate Air (HEPA) filters, or their equivalents. (These filters are at least 99.97 percent efficient for particles 0.3 microns and larger.) Transfer of the canister to the Earth return vehicle is to be done in such a way that no martian materials are transferred to the Earth Return Vehicle except those contained within the sample canister itself. Upon return to the Earth the sample canister must be opened in a facility with the equivalent of BSL-4 containment. Unsterilized samples can be removed from containment only if and when it has been demonstrated that the samples do not contain a biological hazard. In the interim, only sterilized samples are to be distributed to the science community for analysis.

2.3 Hazard Assessment

Direct hazard assessment experiments, involving the exposure of martian materials to different biological entities, are to be done on nonsterilized samples in the containment facility soon after receipt of the samples. Geochemical characterization of the samples and life-detection experiments, which have the potential for providing additional insights with respect to the hazard potential of the samples, should be conducted in parallel with the hazard assessment experiments. Results from early life-detection experiments on unsterilized samples and from geochemical characterization of sterilized samples, together with the results from the biohazard assessment tests, will indicate whether the unsterilized samples can be distributed to the science community or whether further, more definitive, hazard assessment tests are needed.

2.4 Maximize Science Return

A guiding principle behind our recommendations on science analysis is that, **as far as is possible, sample analysis should be done in a distributed manner by the science community at large through the normal NASA Research Announcement (NRA) and peer review process, and not at a central research facility.** Much of the early geochemical analysis should be done on a subset of the samples that have been sterilized in such a way as to preserve most of the information inherent in the samples.

A Preliminary Examination Team (PET) will have the responsibility for performing those analyses that are necessary in order to intelligently allocate and distribute the sterilized subsamples to the science community, and those samples that must be analyzed at the facility because of time or containment requirements. Most of the early science analysis should be done on distributed sterilized samples, and an international panel of scientists should evaluate proposals and allocate samples in light of the findings of the PET. Until the samples are found to be harmless, the bulk of the samples would remain preserved behind a biobarrier. At this stage some preliminary life-detection experiments may be conducted behind the biobarrier at the receiving facility. The design of definitive life detection experiments will likely depend on the results from analyses done on the sterilized samples. Armed with this knowledge, additional proposals for definitive life detection experiments could then be evaluated and conducted on unsterilized samples. Until the samples are proven to be harmless, analyses on unsterilized samples must be done behind a biobarrier, although not necessarily at the sample receiving facility. We do not rule out transport of contained samples to other facilities, provided they have the appropriate containment and controls.

2.5 Alternative Approaches Considered and Rejected

Two alternatives to the direct return of unsterilized sample were considered. The first is to return the samples to the International Space Station (ISS) for initial hazard assessment. The second is to sterilize the samples at Mars before returning them to Earth. Both these alternatives were rejected for the reasons given below.

2.5.1 Return Samples to Space Station

Return to the Space Station (DeVincenzi and Bagby, 1981) might seem like an obvious solution to sample containment, and prevention of any uncontrolled release of martian materials on Earth. However, closer examination indicates that this approach is not only impractical, but may be riskier than direct return of samples to Earth for the following reasons:

1. The Space Station is in low Earth orbit (LEO). To rendezvous with it, the sample canister would have to be placed in LEO. Injection into the appropriate orbit could be done either propulsively or by aerobraking using the Earth's atmosphere. In either case failure could result in uncontrolled entry into the Earth's atmosphere. Similarly, loss of control of the Earth return vehicle while in LEO could result in decay of the orbit and eventually uncontrolled entry. In contrast, the direct return sample canister can be specifically designed to safely enter the Earth's atmosphere and land at a pre-designated target.

2. Even if the sample were safely delivered to the ISS, failure of the ISS itself could result in abandonment by the crew in the Crew Return Vehicle or Shuttle, followed by orbit decay of the ISS and a Skylab-like re-entry.

3. The facilities and personnel available in the ISS to do the necessary hazard assessment and life detection experiments would be far inferior to those available on the ground, and the assessments would be correspondingly more uncertain.

4. Testing for hazards on the ISS would be done in a microgravity environment. Failure to detect hazards might result from failure of the putative organisms in the samples to multiply in this environment. In addition, ambiguities could arise because of inability to discriminate harmful effects of the samples on test materials from harmful effects of the microgravity environment. Our concern is with potential harmful effects of the samples in the terrestrial environment, and this is where the testing should be done.

5. Severe restrictions on power, space, and instrumentation, as well as the difficulty of designing containment and laboratory facilities to operate in a microgravity environment, present a variety of poorly understood problems. The costs of converting the ISS into an appropriately equipped sample receiving facility are likely to be so large as to be impractical. As a consequence, if return to the ISS were a requirement, there likely would be no sample-return. In contrast, facilities exist on Earth that routinely handle hazardous biological materials. It is safer to bring the samples to Earth where facilities appropriate to the unique needs of sample-return can be built.

6. Use of the ISS for evaluating returned Mars samples would usurp other uses of the facility, which is already over-subscribed.

7. Sickness among the astronauts would create several dilemmas, aggravated by the limited medical facilities on ISS, including whether the samples caused the sickness, what countermeasures should be taken, and what the fate of the astronauts should be.

2.5.2 Return Only Sterilized Samples

The following considerations led the panel to reject sterilizing the samples at Mars, or on the way back to the Earth, the main reason being the difficulty of guaranteeing sterilization without destroying much of information inherent in the samples.

1. As discussed in Section 1.3, the chances that there are living organisms in the sample are very low. This is particularly true for samples collected at the surface and not specifically targeted to more favorable environments, such as active hydrothermal areas, if they exist. Even if the samples contain living organisms, the chances that they could survive on Earth and have large-scale pathogenic or ecological effects are low.

2. We do not know how best to sterilize martian materials at Mars or in the space environment, and particularly how to guarantee that sterilization has actually been achieved.

   We are recommending that unsterilized samples be returned to Earth. Only sterilized samples will be distributed to the science community until the samples have been demonstrated to be harmless. Gamma-ray radiation will likely be the method of sterilization since megadoses can be given to the sample without seriously jeopardizing the scientific value of the samples. However, it may not be possible to use this technique at Mars because, to guarantee sterilization, the samples must be exposed to more than 30 Mrad of radiation (Allen, 1999). This would require the launching of a large amount of some radioactive material. Chemical sterilization is ruled out because of the unacceptable levels of chemical alteration that the samples would sustain. If we were to sterilize samples at Mars, it would almost certainly have to be heat sterilization. The temperature required for sterilization depends on the time that the temperature is maintained. Preliminary experiments on soils by Hochstein et al. (1974) showed that sterilization was achieved with 200°C for 24 hours but not with 125°C for 120 hours or with 150°C for 24 hours. Similar experiments by Labeda et al. (1975) showed that soils were sterilized at 200°C for 24 hours but not with 180°C at 30 hours. If we assume that the putative martian microbes have characteristics similar to terrestrial microbes, then these and other experiments (see Appendix A) suggest that conditions similar to 200°C for 24 hours, or more severe, will likely be required to have a high probability that the martian soils and rocks are sterilized.

3. Heat sterilization will destroy much of the information inherent in the samples.

   The samples are expected to contain three types of information: geologic, climatologic, and biologic. Geologic information on primary igneous processes would be the least affected by heat sterilization because the information is stored mostly in high temperature minerals, which would be largely unaffected by being exposed to sterilization temperatures. It could be argued, however, that we already have samples of primary igneous rocks in the martian meteorites so that this aspect of the samples may be of lesser interest than other aspects.

Primary igneous rocks may be altered as a result of weathering at the surface or circulation of groundwater. If they are altered as a result of weathering at the surface, the alteration products retain information about the surface conditions under which weathering occurred. If they are altered as a result of groundwater circulation, the alteration products retain information on the subsurface conditions at the time of alteration. In either case, the products are of considerable interest for their climatic implications and for implications that they might have for conditions under which life may have existed or under which pre-biotic evolution might have occurred. The most heated discussions of ALH84001 have, for example, been not about the primary mineralogy, but about the conditions under which secondary alteration occurred. Alteration products will be low temperature minerals such as clays, zeolites, sulfides, carbonates, halides, and iron oxides and hydroxides. All are susceptible to irreversible alteration at or near sterilization temperatures. In addition, isotopic exchange reactions become significant for clay minerals and related minerals at temperatures above 100°C. Isotopes, particularly oxygen and hydrogen, are important for understanding the physical conditions under which mineral deposition took place, for deciphering how the atmosphere evolved, and for gauging the extent to which atmospheric species interchanged with the surface. (For a detailed assessment of the mineralogical effects of heating martian samples to sterilization temperatures see Gooding, 1990, pp. 16–18.) A major casualty of sterilization is, thus, likely to be our ability to determine the conditions under which alteration occurred.

Clearly, characterization of any organics within the returned samples is central to the search for past or present life. Considerable effort is to be made to obtain drill cores from rocks in order to obtain samples that are minimally affected by the oxidizing conditions right at the surface. Preservation of organics will be strongly influenced by conditions used for sterilization (e.g., times of exposure to various temperatures), so the sterilization regime chosen will have a major impact on the mission success. Preliminary experiments on terrestrial soils indicate significant losses of total amino nitrogen (40%) with 24 hours at 200°C and almost complete loss (80–90%) at 275°C. Selective recemization occurred at 150°C and was almost complete at 275°C. Heating of the Murchison meteorite to 150, 200, and 275°C resulted in alteration of the amino acid content in all cases, and at 275°C alteration was so severe as to leave little useful chemical information (Hochstein et al., 1974).

Soils will be particularly sensitive to alteration by heating. They are likely to be predominantly weathering products that formed during some early era when surface conditions were more favorable to weathering (and life) than present conditions. Information on these earlier conditions may therefore be present in the soils. Because they are probably composed mainly of low-temperature silicate minerals, salts, and possibly ices, they would be especially vulnerable to alteration by heating as discussed above. In addition, there are trace quantities of an oxidant. We know from the Viking biology experiments (Klein, 1978) that the activity of the oxidant declines irreversibly over the 50–125°C temperature range, so that sterilization at temperatures above 125°C would effectively rule out identification of the oxidant. Finally the experiments of Hochstein et al. (1974) suggested that heating of soils to 150°C for 24 hours caused significant morphological changes and formation of artifacts that might be misidentified as biologic in origin.

Thus, while heat sterilization would have a minimal effect on the petrologic value of the samples, their value as indicators of near-surface conditions on the planet in the past and of possible biologic activity would be severely jeopardized.

4. Potential hazards in the samples cannot be evaluated without non-sterilized samples here on Earth, yet must be settled before human exploration is undertaken.

5. Engineering solutions exist whereby completely contained samples can be returned to Earth with a very high probability that containment will not be violated.

6. Even if samples were sterilized on Mars, we would still have to take the appropriate precautions to prevent any uncontrolled release when they are returned to Earth and we would still have to conduct biohazard assessment experiments in a containment before they could be distributed

In summary, sterilization at Mars was rejected as an option because we do not know how to guarantee sterilization at Mars without destroying the scientific value of the samples, nor do we know how to verify that sterilization has been achieved. Search for past or present life and evaluation of former climatic conditions under which life may have started are prime objectives of the Mars exploration program, yet these objectives are placed most in jeopardy by sterilizing the samples prior to Earth return. If non-sterilized samples are returned to Earth, we can use radiation techniques, that minimize damage to the samples, on some subset distributed to the science community, while retaining a pristine sample for determination of those properties affected by the sterilization procedures. Moreover, we can do far more comprehensive testing on the samples here on Earth than we can at Mars in order to assess any potential harmful effects.

2.6 Risks of Returning Unsterilized Samples

The panel adopted the strategy of returning contained, unsterilized samples directly to Earth because (1) the risks that the samples contain anything harmful is very low, (2) containment can be engineered so that the probability of breaching containment is low, and (3) the alternatives have major drawbacks, as just described. It is almost impossible to put numerical values on the risks. For the samples to present a serious threat, they must contain a viable organism that has the following characteristics:

1. Be able to reproduce.

2. Survive acquisition, containment, and return to Earth.

3. Be released because of containment failure.

4. Be transported after release, such as by air currents or biological carriers.

5. Survive the chemical and physical conditions it encounters on the way to a favorable environment.

6. Serendipitously encounter a favorable physical environment or biological host.

7. Survive the host's defenses or the terrestrial biological competition in a favorable physical environment.

8. Thrive and multiply to produce an observable, deleterious effect.

9. Be resistant to eradication countermeasures available in the year 2008.

The panel could see little basis for assigning numerical probabilities to the above items, except for item 3 (over which we have some control), but for the reasons outlined in section 1.3, the probabilities are likely to be small. The largest uncertainty is whether the samples contain any viable martian organisms. However, even if viable martian organisms are present, the chances of them being pathogenic or causing widespread ecological disruption are very small, as was concluded by the NRC Task Group (National Research Council, 1997), and discussed in section 1.3 above.

3 SAMPLE ACQUISITION

3.1 Overview

At the time of writing this document in early 1999, plans for acquiring martian samples were undergoing review. The following is a brief description of the plan at that time (Figure 3-1). Two sample acquisition missions were planned, one to be launched in 2003 and a second to be launched in 2005. Each of these missions was to include a rover that would collect samples in a cache and deliver the cache to a canister, loaded onto a Mini-MAV (Mini Mars Ascent Vehicle) that would deliver the samples to orbit around Mars. The 2005 mission would also include an ERV (Earth Return Vehicle) that would rendezvous with one or both of the sample canisters in orbit around Mars and return them to Earth. The rendezvous with the sample canisters is planned to take place early in 2007. The ERV would leave Mars orbit with the samples in mid-2007 and deliver samples to Earth a year later, in mid-2008.

The 2003 and 2005 rovers are to be delivered to the martian surface on a lander. On each mission the rover will leave the lander and travel on several loops tens of meters away from the lander, and deliver samples to the lander after completing each loop. The first loop will be short; subsequent loops will be of increasing size and complexity. The rover acquires samples by means of a mini-corer, a device that can acquire rock cores 7 mm in diameter, as long as 2.5 cm, and from depths as great as 5 cm within a rock. It also has provision for acquiring a small number of soil samples. Sample collection will be supported by remote sensing instruments on the lander and by analytical instruments on the rover. The cores are to be stored in pigeon holes within a sample cache on the rover. The cache is transferred to the mini-MAV on the lander when the rover has finished its looping traverse. In addition, the lander is planned to have an arm with a scoop to collect a contingency soil sample. The possibility of including a drill and coring device on the lander was also being explored. The number of rock and soil samples to be collected is not yet determined but is expected to be close to 30 with each mission. The total sample mass is expected to be in the 300- to 1000-g range.

The following materials, in no particular order, are of most interest for the primary goals of searching for evidence of past and present life and determining the climatologic and geologic histories of the planet:

(1) Unweathered igneous rocks of different types and ages that span the geologic history of the planet

(2) Breccias from large impact basins

(3) Water-lain sediments, preferably those deposited in low energy environments such as lakes

(4) Loose surface material including soil profiles

(5) Salts (carbonates, nitrates, etc.) and water-ice

(6) Atmosphere

(7) Hydrothermal deposits

All these materials are unlikely to be found at a single site so multiple sample-return missions are required.

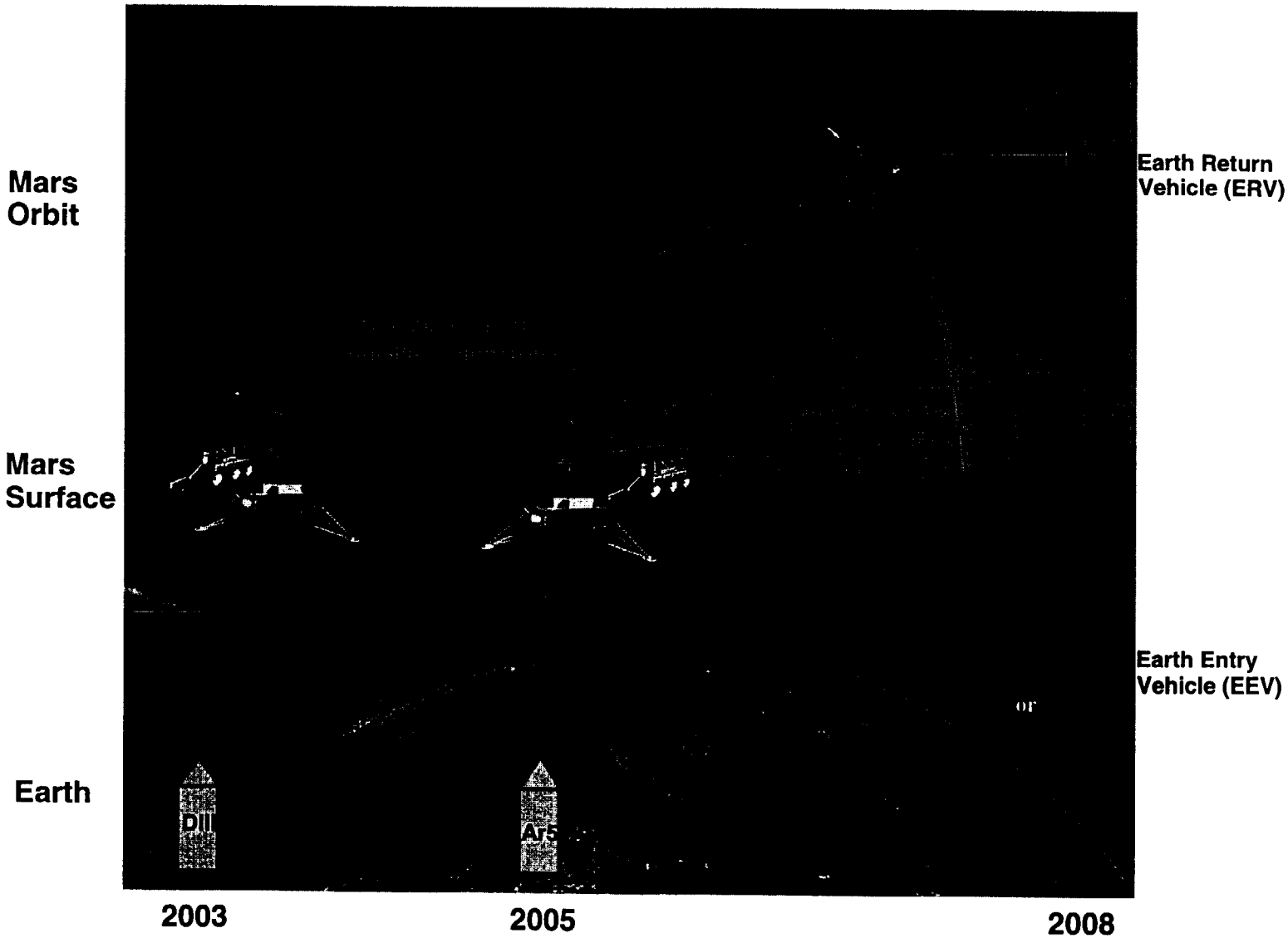


Figure 3-1. Mars Sample Return Missions: 2003, 2005 Opportunities
(Ar5 is Ariane 5, DIII is Delta 3)

3.2 Sample Types

3.2.1 Rock Cores

By the time that MSHARP was convened, the basic strategy of relying mainly on the minicorer for acquiring samples had been adopted. The rationale for using a minicorer is sound. Rocks on the surface of Mars are expected to have a surface weathering rind. We know that the soil is oxidizing, but we do not know how deeply the oxidizing conditions penetrate into rocks. Since weathering can destroy primary rock minerals, structures, and organics that might, for example, contain evidence of past life or past conditions, it is highly desirable to get below the weathering rind. The minicorer will likely penetrate the weathering rind and provide access to the unaltered rocks beneath. The only practical sampling alternative is to collect naturally occurring pebbles or rock chips. However, such small chips are much more likely than rocks and boulders to be weathered throughout. Many of the small pieces at the Viking and Pathfinder landing sites have spectral properties similar to the soil and were probably clods of soil rather than rocks. Taking cores from larger boulders or outcrops also allows the broader geologic context of the samples to be documented. In addition, coring has the practical advantage of providing uniformly shaped samples for packaging. For all these reasons **MSHARP endorses the current plan for acquisition of rock cores.**

The depth of the weathering rind in the rocks could significantly affect the sampling strategy. The rock corer has the capability of extracting two 2.5-cm-long cores from a 5-cm-deep hole. In the most likely event that the weathering rind is only millimeters thick, there is little justification for getting duplicate cores at a single location. We recognize that the Curation and Analysis Planning Team for Extraterrestrial Materials (CAPTEM) has advocated getting two 2.5 cm cores at each sample location and placing both in the same pigeon hole in the sample cache (Appendix B). This was advocated partly to avoid cross-contamination between samples and partly to ensure getting below the weathering rind. We, however, conclude that time and resources may be better spent getting a sample from another rock and adding to the sample variety, rather than getting duplicate samples from the same rock. The total sample mass is very limited and should not normally be consumed in getting duplicate samples of the same rock. Similarly, the number of samples is likely to be limited by the time available to the rover to collect samples, and that time should not normally be consumed in acquiring duplicate samples. The only circumstance that we believe would justify getting two samples from the same borehole is the presence of a weathering rind that is centimeters thick. It may then be necessary to get duplicate cores to get below it. Such an assessment will have to be made during the mission on the basis of observations made on newly acquired cores by the rover instruments. We do not think that cross-contamination is enough of a concern to give up getting different samples in order to get duplicate samples.

3.2.2 Contingency Sample

The lander must acquire a contingency sample, consisting of a scoop of the local soil. This sample would serve two purposes. The first is contingency in the event that the rover is unable to acquire samples and deliver them to the lander. We regard deployment of the rover, moving around the surface, acquisition of rock cores, and transfer of samples from rover the lander all as risky operations. A simple backup plan is needed. Thus, one of the first things that a lander should do on reaching the surface of Mars is load a contingency soil sample in the Mini-MAV.

The contingency sample could also serve a science purpose. The loose material at the surface has intrinsic interest. We do not know how it formed. It may be mostly

weathered material that formed at some time early in Mars history when weathering rates were higher, but other components, such as volcanic debris, are also likely to be present. The presence of duricrust at the Viking and Pathfinder landing sites indicates that the material has undergone further changes in place. It may also contain small, unweathered chips of rock not present in the large boulders and bedrock. Thus, a scoop of the local soil has a science value in addition to providing a backup to the minicorer.

MSHARP, therefore, strongly recommends that a contingency sample be collected by the lander as a contingency against rover failure and to ensure an adequate sample of the local soil. Should core samples (including soils) be successfully acquired, then a decision could be made as to whether or not the contingency sample should be returned.

3.2.3 Soil Samples

The soil is expected to vary from place to place. It may be fractionated by the wind or have undergone different levels of duricrust development. Several samples of soil should, therefore, be collected by the rover in addition to the soil sample collected by the lander. The original Athena proposal for acquisition of samples from a Mars rover (Squyres, 1996) outlined a way of obtaining several soil samples by placing a cylindrical cup within the minicorer and drilling it into the soil to retrieve a sample. The filled cup is then stored in the sample container in the same way as the rock cores. **We support the objective of acquiring multiple soil samples.**

3.2.4 Atmosphere Sample

A sample of the atmosphere is required. The martian atmosphere enclosed in the headspace within the sample canister may satisfy this requirement. The canister must, therefore, be designed so that the atmospheric gases can be extracted when the canister is opened in the sample receiving facility (SRF) on Earth.

3.3 Sampling Strategy

The number of samples collected at each sample site will likely be limited to less than 30 by rover/lander lifetimes and by the time it takes to move around the surface and do the drilling to acquire the samples. Every effort should be made to maximize the variety of samples taken and their total mass. The distribution of rocks at a landing site is likely to be such that one rock type dominates. The second most abundant rock is likely to be much more abundant than the third most abundant, and so forth. A random sampling would likely result in a limited variety or no variety of rock types. Remote sensing data from the rover, lander, and orbiters must, therefore, be used to detect differences in the accessible rocks and to act as a sampling guide so that as many of the different types of rocks present at a site are collected as is practical. Obtaining maximum variety is crucial, irrespective of what we might think causes the variety. For instance, the martian Antarctica meteorite (ALH84001) has given the best hints of life so far in a martian sample, yet a pyroxenite would not normally be judged the best place to look for evidence of life.

Elaborate documentation of samples with in-situ analyses, while desirable, particularly to confirm if a rock potentially available for sampling is different from any previously samples, should not be done at the expense of acquiring additional samples. (Fortunately, most of the in-situ analyses desirable to document the samples can be done at night when other operations are not possible, so that documentation can be done with minimal impact on sample acquisition). A possible exception to the general conclusion that in-situ

documentation should not be done at the expense of getting more samples is determining the thickness of the weathering rind early in the sampling phase, as indicated in Section 3.2.1.

3.3.1 Sample Separation

We do not consider cross-contamination between samples to be a major concern. All the rock samples will inevitably be contaminated with soil. It is simply impractical to keep all the various components that will come into contact with the samples free from martian dust, which is present not only on the ground, but also in the atmosphere. Cross-contamination between rocks is similarly of minor concern. We can see little penalty in putting two different rock samples in the same pigeonhole in the sample container. We note, however, that CAPTEM advocates taking measures to prevent cross-contamination (Appendix B).

4 PREVENTION OF SAMPLE CONTAMINATION

4.1 Overview

The collection and return of samples includes several transfers, any one of which might cause contamination (Figure 4-1).

We are specifically concerned with three forms of contamination:

(1) Forward contamination of Mars by terrestrial organisms and by terrestrial organic matter associated with intact cells or cell.

(2) Contamination of samples with any terrestrial materials, both organic and inorganic.

(3) Back contamination, or the introduction of extraterrestrial materials into the terrestrial environment.

Back contamination, which is of the greatest public concern for sample-return missions, is controlled by containment and handling protocols and is dealt with in Sections 5 and 6. This section deals with items (1) and (2) above, which are primarily scientific concerns.

Forward contamination of Mars was of concern to the committee only insofar as it affected the integrity of the returned samples (and supporting in-situ observations). Planetary protection policies for Mars missions have been revised since the time of the Viking missions (National Research Council, 1992). Cleanliness and sterilization requirements depend on the classification of the mission. We assume that components that land on the martian surface but do not return to Earth (rover, lander) fall under category IVa for planetary protection purposes (DeVincenzi et al., 1996). That is, they are landers without life detection experiments. For components that are returned to the Earth, the greater concern is not forward contamination of Mars but back contamination, for which there are the more stringent Category V requirements, i.e., sterilization of all spacecraft components returned to Earth that have been in contact with martian materials, and containment of the samples (National Research Council, 1997). We are also advocating cleanliness and sterilization requirements more stringent than those needed for Class IVa for some hardware that does not return to Earth, because of their potential for contaminating the samples.

Terrestrial contamination of the samples is of considerable concern. Potential contaminants include living organisms, dead organisms, cell debris, organic molecules, and inorganic compounds. They could result during the mission from interchange between various spacecraft components and the samples, or subsequent to the mission during sample handling. Round-trip transport of viable organisms is a possibility. Bacterial spores may have survived two and a half years exposure to the lunar environment on the Surveyor 3 lander before being returned to the Earth by the Apollo 12 mission (Mitchell and Ellis, 1972). Some viable seeds and spores were also found on materials returned from the Long Duration Exposure Facility (LDEF) satellite after several years in space (NASA, 1991; Horneck, Bucker, and Reitz, 1991)

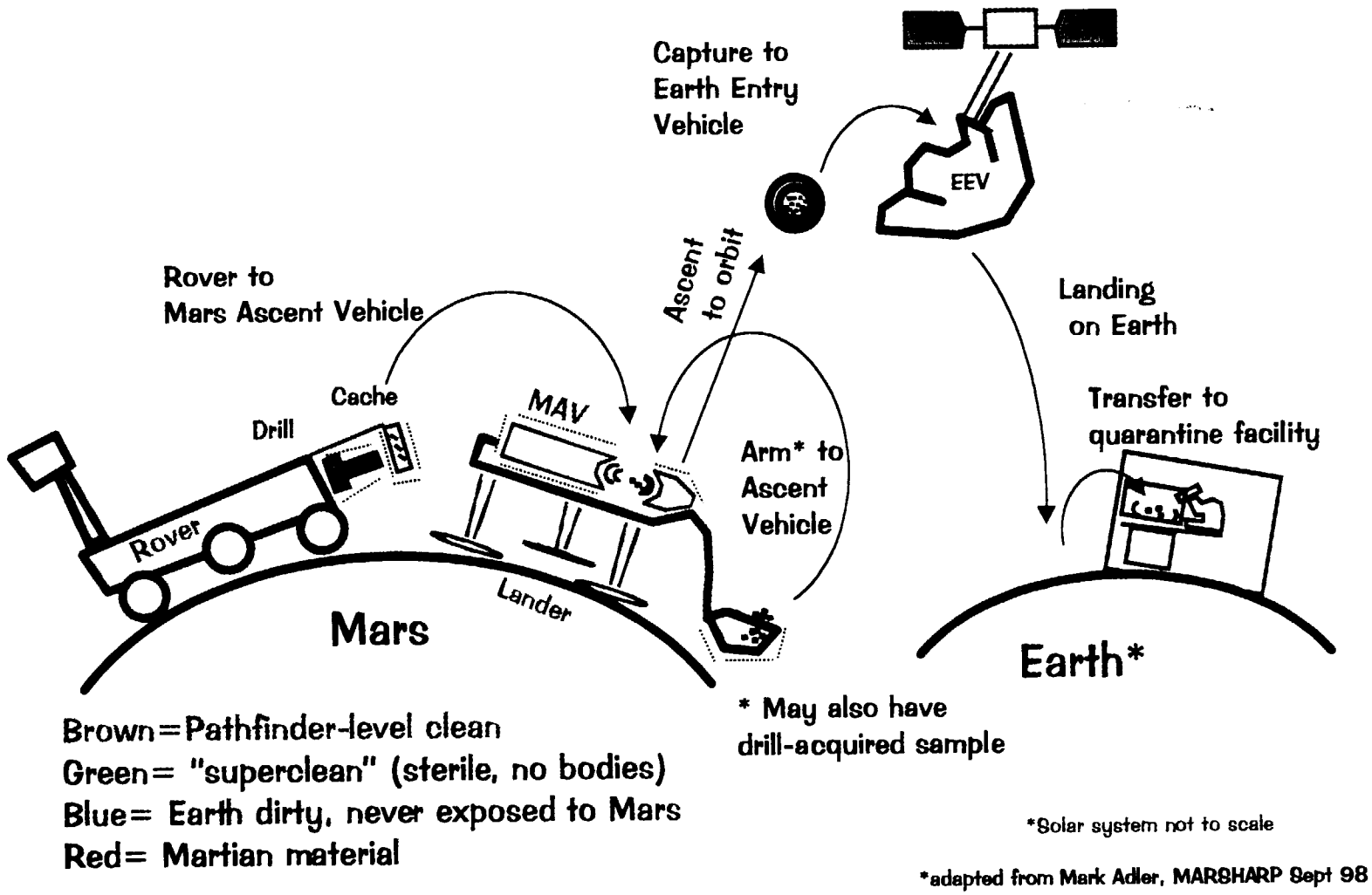


Figure 4-1. Mars Sample-Return Contamination Control

Concern over terrestrial contamination of the samples arises not because such contaminants present a threat, but because they undermine our ability to interpret data from the samples. They will not only place the validity of the scientific results from the samples in jeopardy, but also affect how long they are kept under strict biocontainment. False positives from the hazard analysis or life detection tests (Sections 6.1.3.4 and 6.1.3.5) could indefinitely delay release of unsterilized samples to the science community.

4.2 Approach

Because the samples will come in contact with, and be in close proximity to, terrestrial materials on the spacecraft, some terrestrial contamination is inevitable. Ideally, we would like to specify that there be no dead or live terrestrial organisms and no terrestrial organic molecules in the sample chamber when it is opened in a containment facility on Earth. But to guarantee this may not be possible, and if possible, it may be prohibitively expensive. Moreover, the cost of failure to achieve absolute absence of contaminants is not catastrophic. There may be some erosion of the science and some extension of the time that unsterilized samples must remain behind a biobarrier. But failure to achieve an absolute cleanliness standard does not present a hazard (as might failure to achieve a containment standard).

The sensitivity of analytical instruments continues to increase with time. Identifying certain organic molecules present at less than 1 part in 10^{12} will be routinely achievable when the samples are returned in 2008. This capability places greater demands on cleanliness. Fortunately, cleaning techniques are also evolving. Techniques such as using hydrogen peroxide plasma or ultra pure water, for example, were not available (and not needed) when Viking was launched in 1975. Thus more stringent contamination controls than were needed by Viking may reasonably be anticipated.

Recognizing that some terrestrial contamination could occur, **we are advocating, that in addition to following appropriate sterilization and cleaning procedures, that assays be made of potential contaminants, and that contaminant tracers and 'witness plates' be used to help identify contaminants.**

4.3 Sterilization and Cleaning

Different cleanliness standards should apply to different spacecraft components according to how likely they are to transmit contaminants to the samples.

4.3.1 Sterilized and Superclean Components

All components of the spacecraft that come in direct contact with the samples must be sterilized and cleaned to significantly higher standards than the rest of the spacecraft. These components include the minicorer, the sample cache, the contingency sample scoop, and the interior of the sample canister. We have opted not to quantify what is meant by "significantly higher standards", because we do not know how difficult and costly they are to achieve. We note, however, that a Planetary Protection Workshop held at JPL Dec 9-11, 1998, suggested that a reasonable goal for these components, is that, in addition to being sterilized, they should be cleaned to about 10,000 times better than Viking and enclosed behind some form of bioshield on the surface of Mars to prevent contamination from other, less stringently cleaned components. (The December 9-11 workshop is to be published; viewgraphs of the meeting are in Carr, 1998.)

4.3.2 Other Components That Land on Mars

All components that go to the martian surface must be cleaned to at least Viking levels of cleanliness prior to sterilization in order to satisfy planetary protection requirements for forward contamination. For Pathfinder this was interpreted to mean less than 300 spores/m2 and less than 10^5 total spores on exposed surfaces. Pathfinder achieved 12 spores/m2 and less than 2.4×10^4 total. **We believe a reasonable goal for the components that go to the martian surface, including the rover, the lander, and the Mars Ascent Vehicle (MAV), is to achieve at least the Pathfinder cleanliness levels.**

4.3.3 Earth Return Vehicle

Because the sample will be enclosed in the sample canister before it is delivered to the Earth Return Vehicle (ERV), terrestrial contamination of the ERV is not an issue. Contamination of the ERV with martian materials is, however, a very important issue (see section 5.4).

4.4 Contamination Monitoring

Given that, even with our best efforts, some terrestrial contamination of the sample could occur, several steps should be taken to aid in recognition of the contaminants.

4.4.1 Tracers

The Athena team, which is largely responsible for design of the sample acquisition system that is to be used at Mars, advocated adding molecular tracers to drill bits to allow subsequent assessment of the extent to which contaminants might have penetrated into the cores (Squyres, 1996). MSHARP discussed this concept in some length and recommended that CAPTEM look into it further. MSHARP and CAPTEM both advised against impregnating the drill cores with a molecular tracer. (The CAPTEM advice was in two letters included in Appendix B; the MSHARP advice is in the minutes of the Mars Sample Handling Panel, January 9, 1998 [Jet Propulsion Laboratory, 1998].)

The suggestion to add tracers to the drilling bit stemmed in part from their successful use in looking for microorganisms deep within the Earth's crust. In these experiments, molecular tracers, such as perfluoromethylcyclohexane and latex beads with fluorescent dyes are added to the drilling mud. The mud is forced part way into the cores. Absence of tracers in the interiors of the cores gives assurance that there is no contamination.

Both MSHARP and CAPTEM concluded, however, that collection of martian samples is only loosely analogous to acquisition of deep drill cores on Earth. Contamination of the martian sample could come from a variety of sources, not just from the drill bits. Tracers would allow contamination from the drill bit itself to be recognized, but would be of little help for other contaminants that might be transferred to the surfaces of the cores after stowage. Moreover, contamination by the drill bit would be recognizable from the tantalum, tungsten, and cobalt in the steel and abrasives that are used in fabrication of the drill bits. The main concern about using molecular tracers on the drill bits is that the deliberate addition of the tracers could significantly interfere with interpretation of data from the samples. Tracer materials that contain hydrogen, carbon, nitrogen, and oxygen, for example, could have significant negative effects on Earth-based analyses. The isotopes of these elements all contain crucial information on the evolution of the atmosphere, past climates, and the potential for life. Carbon and nitrogen are likely to be present in only minute amounts, and deliberate addition of terrestrial carbon and nitrogen could mask the signatures that we are

looking for. **We, therefore, recommend that molecular tracers not be added to the drill bits.**

The above discussion does not rule out all uses of tracers. The main objection to adding tracers to the drill bits is the deliberate addition of organic molecules to the samples when elements in the drill bit themselves could be used as tracers. **There may be justification for use of tracers in other circumstances. Inorganic tracers might be added to some lubricant on the rover, for example, that is unlikely to contaminate the sample, but which, if it did, would need to be recognized. In this case contamination is adventitious, not deliberate, and the tracer would not normally interfere with subsequent sample analysis.**

4.4.2 Witness Plates

We strongly recommend the use of witness plates for monitoring contaminants. Some plates could be exposed to the same cleaning conditions as the rovers and landers here on Earth, then removed as late as possible before launch. Others could be included in the sample cache but sealed before arrival at Mars. Yet others might remain open during sample acquisition and be sealed prior to loading in the MAV, and thus have a high ratio of contaminant to martian material. Witness plates thus could help not only to identify, but also to determine their source. We recognize that incorporation of witness plates into the plan has cost and engineering implications, and we do not know what they are. We are not, therefore, insisting that carrying all types of witness plates be a requirement but that the potential use be aggressively explored.

4.4.3 Assays

As late as is practical before launch, assays should be made of the different types of contaminants present on different spacecraft components. The assays should include not only viable organisms, but also organic compounds and cell debris.

5 TRANSPORT OF SAMPLES FROM MARS TO EARTH

5.1 General Statement

This section deals with concerns about back contamination, beginning with transfer of the samples from Mars and continuing through activities associated with the SRF on Earth. How the samples should be handled after receipt in the SRF is discussed in the next section. The NRC recommendations listed in 1.4.2.1 through 1.4.2.3 above are those that are relevant to transfer of samples from Mars to the Earth. In summary, these recommendations are:

(1) No uncontained martian materials, including spacecraft materials that have been exposed to the martian surface, should be returned to Earth unless sterilized.

(2) If sample containment cannot be verified, the samples should either be sterilized in space or not be returned to Earth.

(3) Integrity of containment should be maintained through re-entry of the spacecraft and transfer of the sample to an appropriate receiving facility.

5.2 Sample Containment

We recommend that the samples be contained to a high confidence level before leaving the martian surface. **We further recommend that introduction of unsterilized martian material into the Earth's environment be kept to a very low probability, predominately through system design using seals and filters, rather than through monitoring containment and incorporating various contingency responses into the design.** Containment is to be maintained until the sample is in an appropriate receiving facility.

In the current architecture, the sample caches on the rover are to be delivered to a sample canister in the mini-MAV and launched into Mars orbit. The Earth Return Vehicle (ERV) must then retrieve the sample canister and place it within the Earth-Entry Vehicle (EEV). **Transfer must be effected in such a way that no martian materials, other than the contained sample, are transferred. The sample canister must be sealed before it makes its rendezvous with the ERV in order to preserve the sample under the conditions at the martian surface, and as a first line of defense against uncontrolled release.** The ERV will then transport the EEV to Earth and deliver it to a target for entry into the Earth's atmosphere. The EEV must be designed such that there is a high probability of maintaining containment during entry into the Earth's atmosphere and during landing.

5.2.1 Nominal Earth Entry Conditions

We believe that the best approach to containment of the martian sample during return to Earth is one that emphasizes the reliability of the containment system itself, rather than a system based on containment monitoring and incorporation of various responses, such as sample sterilization or spacecraft diversion, that might be used in different contingencies. The containment system (Figure 5-1) can be designed to be extremely robust and allow its reliability to be extensively tested before launch. In contrast, monitoring the functioning of a containment system in real time raises reliability issues for both the containment system and a likely complex monitoring system. Continual monitoring of biological containment

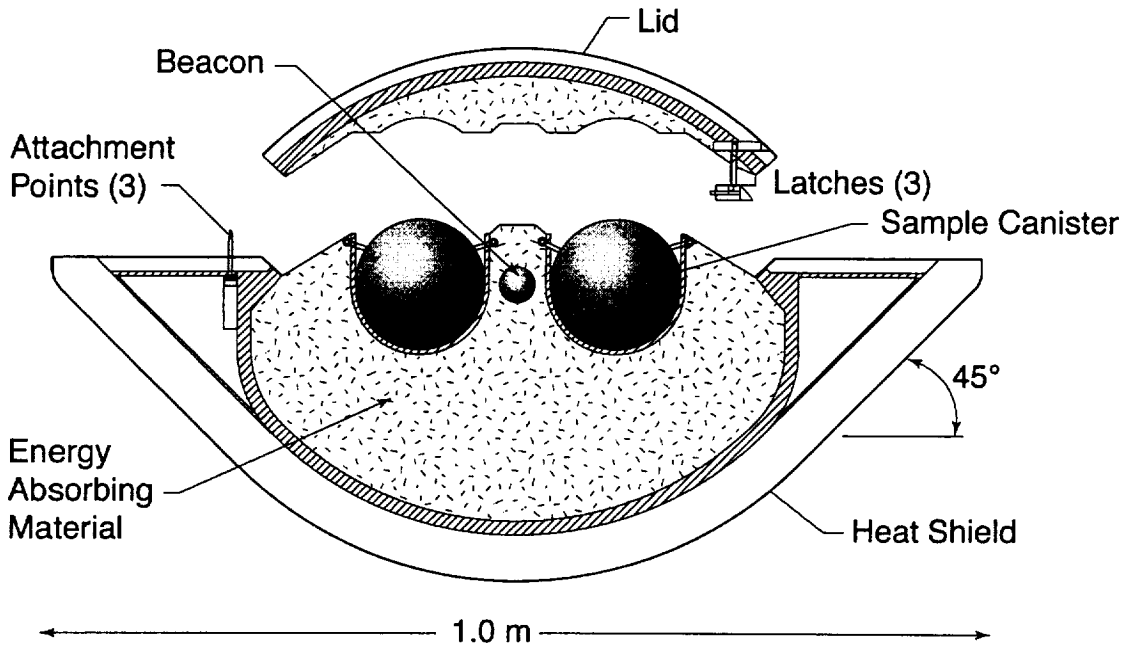


Figure 5-1. Earth Entry Capsule (March 1999 design from M. Adler, JPL)

after sealing and during transit to Earth is difficult to verify in real time. What monitoring techniques could be used? Gas losses from the container might be monitored, but the sensitivities required to ensure complete biological containment may not be practical. Further, if a signal is received signifying containment loss, is that signal real or due to a malfunction of the monitoring system? If a system were included in the EEV to sterilize or destroy the sample in the event of an indication of loss of containment, how does one verify the proper operation of that system? The most likely time for failure of sample containment is upon landing on Earth, when the EEV is subject to its highest g-loading. If containment were to fail during landing, there probably would be insufficient time to consult monitoring systems or to take mitigating steps to counter possible loss of containment. Because of these uncertainties, we conclude that resources are better spent devising a fail-safe containment system rather than elaborate monitoring systems and contingency capabilities.

Long-term isolation of biologically hazardous materials in terrestrial laboratories is managed by both seals and filters. **We conclude that extremely robust containment systems can be built using multiple seals and HEPA filters or their equivalents.** To achieve containment, we recommend that the primary sample container be sealed to high reliability, such as by explosive welding, and be able to withstand high stresses that might be encountered on landing. We recommend further that additional seals and biological filters be incorporated into the re-entry spacecraft to act as additional biological barriers in the unlikely case of primary barrier failure. Thus, HEPA filters could be placed between the different sealed sections, for example, between the sample canister and the rest of the EEV. The combined use of seals and filters may offer the additional advantage of having failure modes that are likely different. Such a dual seal/filter system, in the unlikely event of a primary seal failure, would catch any leaking material in the HEPA filters in the same way that hazardous materials from terrestrial laboratories are prevented from leaking into the environment. Such a design could lead to extremely low probabilities of containment failure (Carr et al., 1998).

While we are advocating against continual monitoring of the seal integrity, we are recommending verification of the primary sealing of the inner sample container at Mars. A one-time verification of this seal might be achieved by optical inspection while on Mars, by verifying that the container still contains martian ambient atmospheric pressure once it reaches the vacuum conditions of orbit, or by some other procedure.

5.2.2 Anomalous Earth Entry Conditions

The discussion in the previous section pertains only to the most probable situation, where the EEV maintains its integrity during Earth entry. Clearly if the EEV enters the Earth's atmosphere anomalously and disintegrates, then the performance of the seals and filters may become immaterial. These low-probability, anomalous events must be modeled to demonstrate that the probability of unplanned introduction of martian materials into the Earth's environment as a consequence of them is very low.

Management of the Earth Return trajectory will be an important element in reducing vulnerability to anomalous Earth entry conditions. The initial trajectory of the ERV should be biased far enough away from the Earth such that failure of the ERV before final targeting of the EEV is highly unlikely to result in encounter with the Earth.

5.3 Contingency Sterilization

The NRC task group on sample-return recommended that if containment cannot be verified, then the samples should be sterilized or not returned to the Earth. As indicated above, **we are questioning the need for a contingency sterilization. If the sealing is successful at Mars, then failure of the seal during return to the Earth is viewed as an extremely low probability.** The most likely times for seal failure are at Mars, when the primary seal is made, and during Earth entry and landing. A capability for in-flight sterilization offers no protection against failure at Earth. The primary justification for contingency sterilization is for use in the event of a seal failure at Mars. Given that resources are limited, they may be better spent in designing more reliable seals, with the understanding that the samples would not be returned in the unlikely event that the seals fail.

5.4 Sample Preservation

Scientific guidelines for preservation of samples collected from Mars are summarized in Table 5-1 from Gooding (1990). The main issue in sample preservation is temperature. Different effects occur at different temperatures; these range from loss of gas to changes in mineralogy. It is difficult to argue that temperatures should be maintained below the maximum experienced on Mars. For the initial sample-return missions, all samples will have been collected within a few centimeters of the surface. For this depth, all samples should have commonly experienced temperatures at least as high as 240 K. There is, therefore, a strong desire to keep the sample below this temperature during cruise, Earth entry, and landing. We recognize that this may be impractical during landing, but since the effects on the sample will depend on the time spent at undesirably high temperatures, we recommend that the system be designed to minimize these times.

Table 5-1. Conditions Recommended for Preservation of Martian Samples by Gooding (1990)

Contamination	For each element, <1% of the concentration in Shergotty meteorite (Rock, sediment, or soil sample) For each element or compound, <1% of the concentration in the Viking lander atmospheric analyses (atmospheric sample)
Temperature	<260 K (igneous rock sample, unweathered) <230 K (soil, sediment, deep regolith, or weathered rock sample)
Pressure (head-space gas)	<1 x 10^5 Pa (1 atm) (igneous rock sample, unweathered) <1 x 10^3 Pa (0.01 atm) (soil, sediment, deep regolith, or weathered rock sample)
Ionizing radiation	5 g/cm^2 shielding
Magnetic fields	<5.7E–0.05 Tesla (1 Earth field)
Acceleration/Shock	<7 g (1 g = 9.81 m/s^2)

5.5 Aseptic Transfer

No martian materials other than those contained within the sample canister can be returned to Earth. In order to prevent delivery of martian materials to the Earth on spacecraft components, the cache containing the samples must be sealed on the martian surface and delivered to the Earth Return Vehicle (ERV) without contaminating the vehicle with martian materials.

6 SAMPLE HANDLING ON THE GROUND

6.1 General Approach

Consistent with the recommendations of the NRC Task Group, the samples are to be assumed hazardous until proven otherwise. This requires that the sample canister be returned to, and opened in, a containment facility that protects the Earth's environment and inhabitants from potential hazards. The facility must also protect the samples from terrestrial chemical and biological contamination. Portions of the sample may be removed from containment for additional studies, but only if sterilized first.

Three types of analyses will be done on the samples:

(1) Hazard analysis, in which the potential of the samples as a threat to the terrestrial environment and its inhabitants is assessed.

(2) Life detection, in which evidence for past or present life is assessed.

(3) Geochemical characterization directed at better understanding the evolution of the planet.

The three types of testing are not mutually independent. The assessment of whether the samples could be hazardous will, for example, depend on life detection testing and on various geochemical characteristics of the samples, such as the organic carbon content.

A guiding principle behind the procedures for handling martian samples should be to use the best available technology and expertise. We also anticipate that there will be considerable public interest in expeditiously determining if the samples present a hazard and if any form of life is present. **In practice, comprehensive, rigorous testing may be difficult or impossible to accomplish at a single facility. To accomplish the planetary protection and scientific goals, we should capitalize on the diverse analytical capabilities and expertise of the scientific community at large. We are recommending, therefore, that soon after receipt of the samples (after they have been classified, examined, and imaged for archival purposes) a small subset of the samples be sterilized and distributed to the science community for geochemical characterization, while other analyses, mainly hazard assessment and life detection, are being conducted behind the biobarrier. It may also be desirable to send contained samples from the Sample-return Facility to other, well instrumented biosafety facilities, such as Center for Disease Control (CDC), using well established procedures for transporting biohazardous materials, but with special attention to avoiding terrestrial contamination.**

The results of the preliminary life detection and hazard experiments, combined with early findings on the sterilized samples may be sufficient to declare the samples nonhazardous, in which case the samples could be removed from behind the biobarrier to a curation facility, as they were during the Apollo program. In the more likely case that the results of the preliminary tests are inconclusive, more definitive tests would then be designed using the knowledge gained from the analyses already completed. This approach allows geochemical characterization by the science community at large to proceed while preliminary hazard detection and life detection testing is being done. Definitive hazard analysis and life detection testing may be a lengthy process, particularly if there are positive indications, either real or false, in the early testing. Figures 6-1 through 6-3 indicate how the samples should be handled and some of the issues that must be addressed at each stage.

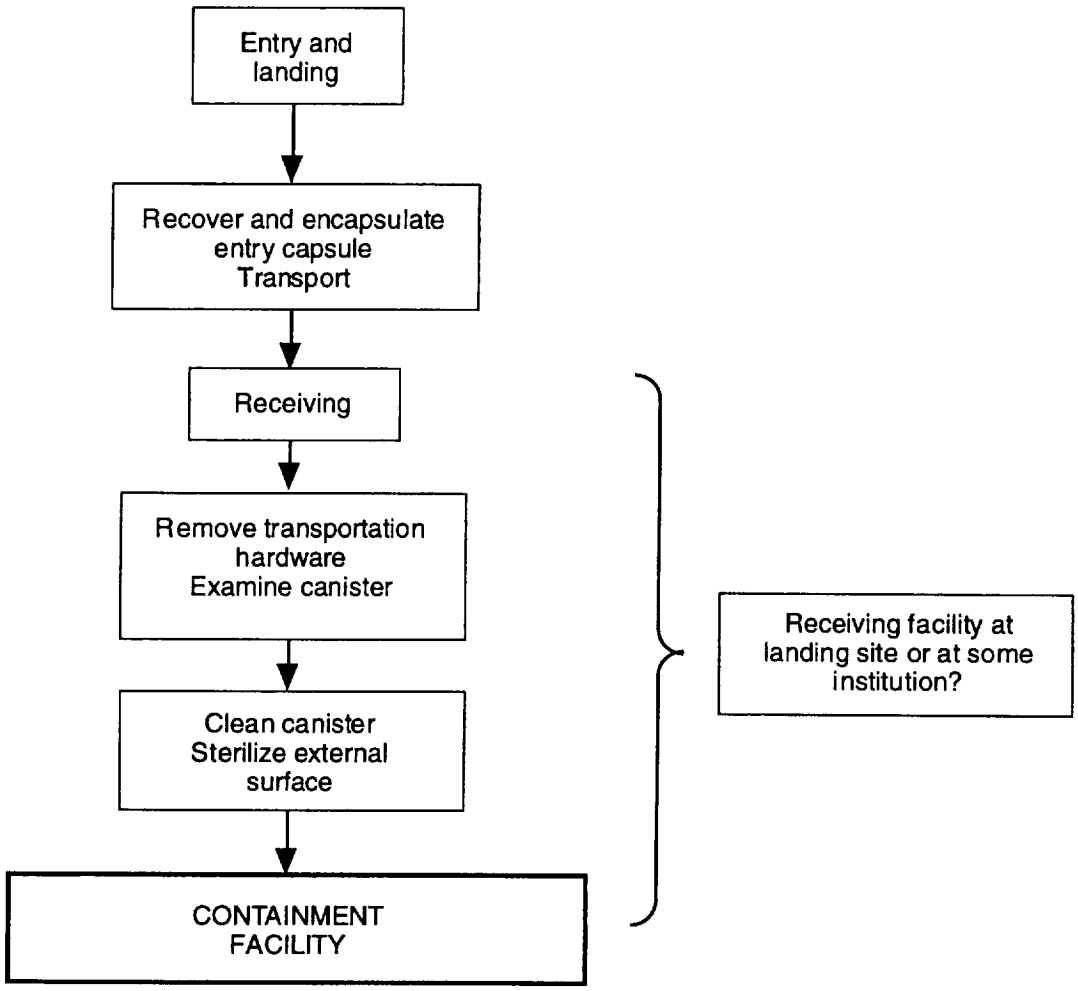


Figure 6-1. Delivery of the Samples to the Containment Facility

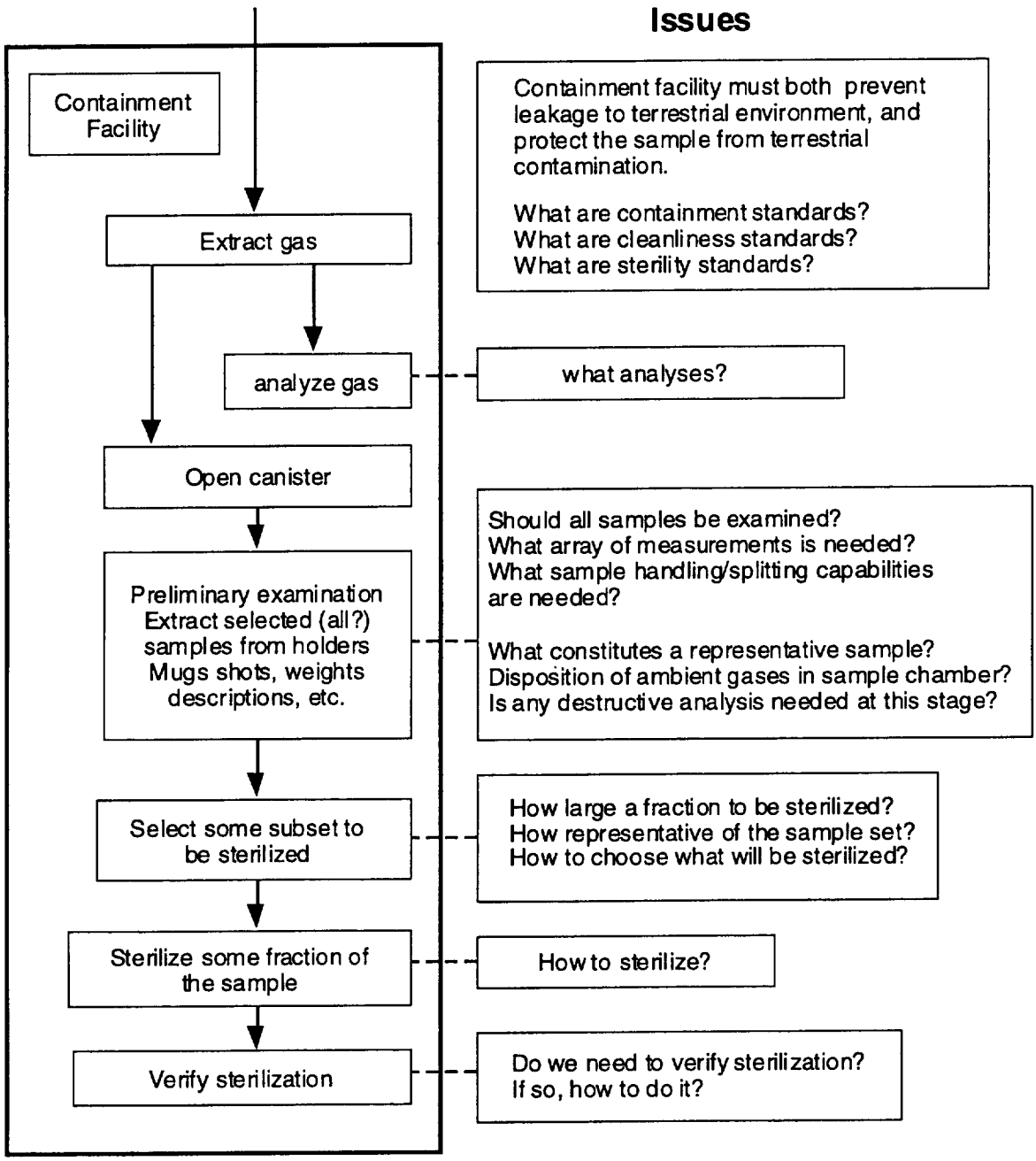


Figure 6-2. Early Processing of Samples at the Containment Facility

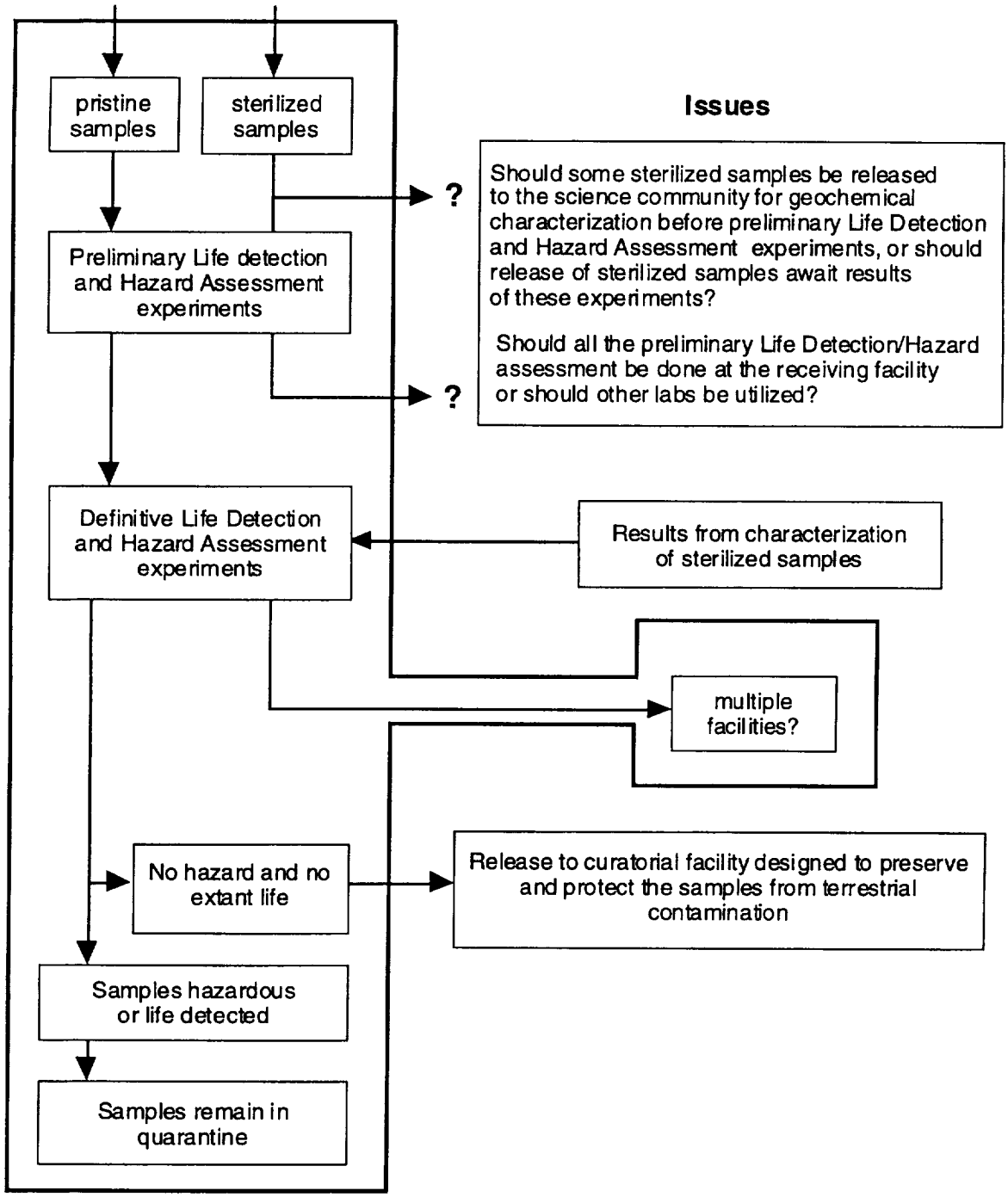


Figure 6-3. Later Sample Processing at the Containment Facility

6.2 Apollo Experience

During six sample-return missions of the Apollo program, 382 kg of lunar rocks and soil, comprising 2196 individual specimens were returned. In contrast the first sample-return mission from Mars is expected to return a few to several hundred grams of rocks and soils, comprising less than 50 individual samples. To handle the lunar samples, an 8000-m^2 Lunar Receiving Laboratory was built at Johnson Space Center at a cost of roughly $130 M in 1998 dollars. At the height of the quarantine phase of the project, 200 technicians worked three shifts a day to support 100 NASA civil servants and visiting scientists. At the conclusion of the program, all the samples were moved to a facility more suitable for sample curation and preservation. A building specially designed to keep the samples pure was completed in 1979 and has been operated since with a staff of less than 20 people (Allton, 1996).

For Apollo, an Interagency Committee on Back Contamination (ICBC) advised NASA on quarantine procedures. Testing for toxic and infectious agents was done behind a biobarrier by a Biosciences and Containment Group. A Preliminary Examination Team (PET) performed the initial geologic and geochemical examination of the samples in the quarantine facility, and a Lunar Sample Analysis and Planning Team (LSAPT) was concerned primarily with sample preservation and allocation, functions now performed by the CAPTEM.

A major difference between Apollo and return of Mars samples is that Apollo had to be concerned with potential effects of the lunar samples on the crews. This affected facilities design, experimental protocols, and the time within which quarantine issues had to be resolved. A goal of the testing was to provide safety clearance for lunar samples within a period of approximately 30 days (NASA 1965 Summer Conference on Lunar Exploration and Science). With the Mars samples, there is much less need for rapid validation that the samples are safe for distribution, particularly if sterilized samples are released while validation is underway.

6.3 Sample-Handling Facilities

Our perception of the sample-handling facilities differs somewhat from that enunciated by the TGISR, and this is in part enabled by the approach described in section 6.1. **The sample-handling facilities should mainly serve the science community rather than do independent research.** The main functions of the facilities are to keep unsterilized samples isolated from the terrestrial environment until they have been demonstrated to be harmless, to do whatever testing is needed to evaluate whether the samples are harmless, and to get well preserved samples out to the science community safely and as expeditiously as possible. While these are the main functions, some additional tasks, which are research in nature, may need to be done because they must be done immediately upon receipt of the samples and/or because they must be done behind a biobarrier.

The sample-handling facilities can be viewed as consisting of two components: a sample receiving facility (SRF) and a sample curation facility (SCF). The two facilities could be in separate locations, but there is overlap in functions between the two and they need not be two separate physical entities.

The SRF should have the following functions:

(1) Prevent uncontrolled introduction of martian materials into the terrestrial environment.

(2) Preserve the integrity of the samples by minimizing alteration and terrestrial contamination.

(3) Make an inventory of the samples and perform those analyses on the samples that are needed to intelligently select samples for sterilization and early distribution to the science community.

(4) Perform selected sample analyses.

(5) Test for any possible hazards within the samples.

(6) Sterilize some subset of the samples.

(7) Maintain accurate historical records on the samples.

The SRF must be designed both to contain the samples behind a biobarrier and to preserve the samples from terrestrial contamination. **Such a facility does not yet exist to our knowledge.**

If the samples do not contain a biological hazard, then they and any remaining sterilized samples would be transferred to a sample curation facility (SCF) whose functions would be to:

(1) Preserve the samples.

(2) Classify and document the samples, and make the information publicly available.

(3) Prepare and distribute samples for research and education.

(4) Maintain historical records of all the samples.

The curatorial facility at JSC is designed specifically to perform these functions for NASA for extraterrestrial materials in general. We see no reason why it should not do the same for Mars, although modifications may be needed in order to appropriately preserve martian samples.

6.4 Sample Receiving Facility

6.4.1 SRF Functions and Their Implementation

Some of the capabilities of the SRF and some of the procedures that might be followed in order to accomplish its task are described below under the separate functions listed above.

6.4.1.1 Prevent Uncontrolled Introduction of Martian Materials into Terrestrial Environment. Until it has been demonstrated that the samples do not present a hazard, all nonsterilized samples must remain behind a biobarrier. No quantitative standards exist for different levels of biocontainment; containment levels are specified in terms of the procedures that are followed.

"The term 'containment' is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure to laboratory workers, other

persons, and the outside environment to potential hazardous agents." (CDC-NIH Manual, 1993).

There are three elements of containment: laboratory practice and technique, safety equipment, and facility design. In the biomedical community, the risk assessment of the work to be done with a specific agent determines the appropriate combination of these elements. Although a putative martian entity (if any exists) in returned materials would be considered an unknown biological agent, the CDC-NIH guidelines are still applicable and appropriate.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to hazardous agents, is provided by good microbiological techniques, biobarriers, and the use of appropriate safety equipment. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious material, is provided by a combination of facility design and operational practices.

The CDC-NIH manual (1993) describes four biosafety levels (BSLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, for the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. Containment is typically maintained by use of HEPA and ultra low penetration air (ULPA) filters, by managing pressures within the containment area, and by ensuring that all tools, containers, materials and equipment are removed from the containment area only through sterilizer transfer locks. How containment is effected and adapted for martian samples will depend on what processing and analyses must be done behind a biobarrier. Some possible alternatives that have been suggested include:

(1) Build a BSL-4 containment laboratory in which all personnel wear biological barrier suits. Townsend (1990), for example, recommended building a 370 m^2 (4000 ft^2) BSL-4 Biological Testing Complex to handle Mars samples.

(2) Do all the processing and analyses within interconnected Class III Biological Safety Cabinets, with sealable doors between cabinets, all contained within a 'high-end' BL-3 containment laboratory. (Recommendation of the Containment Subgroup of the Mars Quarantine Protocol Workshop, NASA/ Ames, June 4–6, 1997).*

(3) Acquire a number of commercial, modular, CDC-certified, biocontainment laboratories and ship them to an appropriate location. After the results of the early analyses are completed, expand or contract the modular laboratory as needed.

(4) Capitalize on existing, well instrumented BSL-4 facilities (as at CDC) and either locate the receiving laboratory adjacent to them or, for some testing, ship selected samples to these facilities, following well-established procedures for the shipping of hazardous biological materials. We acknowledge that some existing facilities might be used for some specialized testing because of their unique combination of containment and instrumentation. Existing facilities are, however, unlikely to be usable for the general handling of martian samples, unless considerably modified, because they do not have the appropriate controls for terrestrial contamination, as listed below under 6.3.1.2.

*Draft in review: DeVincenzi, D.L., et al. (editors), 1998. *Proceedings, Mars Sample Quarantine Protocol Workshop*, NASA/Ames, June 4–6, 1997, Preliminary draft. NASA/Ames internal document, 6/12/98.

The possible approaches described above are not mutually exclusive. A modular BSL-3 lab with Class III safety cabinets could be used in conjunction with existing BSL-4 facilities. While we have not explored in depth how the SRF should be configured, **we are skeptical of the need for a complex facility of the type described in (1) above, and used during the Apollo mission. We believe that all the applicable biocontainment requirements can be met in a modest-sized, modular receiving facility with BSL-4 areas, coupled with distribution of sterilized subsamples to the science community. If it is deemed appropriate, nonsterilized samples can be shipped to other BSL-4 facilities, using established procedures for shipping biohazardous materials, in conjunction with the necessary measures for avoiding terrestrial contamination.**

6.4.1.2 Preserve Sample Integrity. The main concern here is protecting the samples from terrestrial chemical and biological contamination. Secondary concerns are cross-contamination between samples and degradation of the samples by being maintained at conditions that differ from Mars ambient conditions. Control of terrestrial contamination requires that all gases in contact with the samples be filtered. Air coming into the SRF must be filtered for contamination control; the outgoing air must be filtered for containment. Similarly all tools, reagents, and surfaces must be sterilized and cleaned.

In order to minimize terrestrial contamination, the following should be considered (extracted and paraphrased from Townsend, 1990):

(1) It is essential to provide controls and identify materials used in the construction of all tools, equipment, containers, and environmental cabinets used in handling, processing, and storage of samples. Many construction materials were considered for the Apollo program, but were narrowed down to steel, aluminum, and Teflon. These materials have proven effective over the years in protection of the lunar samples.

(2) No plastics of any kind, except the unplasticized fluorocarbon Teflon should be used, except in gaskets that would not have contact with the samples.

(3) The most undesirable contaminants are Pb, U, Th, Li, Be, B, K, organic compounds, and microorganisms (living or dead). Other undesirable contaminants are Rb, Sr, noble gases, and rare earths.

(4) There must be provision for individual samples with apparent differences to be packaged separately and hermetically sealed to prevent particulate, gaseous, and biological cross-contamination.

(5) All tools, containers, and equipment used to handle and process Mars samples must be sterilized, cleaned, and packaged according to approved cleaning procedures, such as Graf (1971), and be introduced into the containment area only through a sterilizer transfer lock.

Long-term storage of the samples should be under a noncontaminating gas (such as nitrogen) and at temperatures as close to the 240 K as is practical.

6.4.1.3 Sample Inventory. The SRF must separately package, inventory, and make a preliminary characterization of all the samples. Characterization will include weighing, photographing, and preparing geologic descriptions. It could include nondestructive analyses such as x-ray fluorescent spectrometry (XRFS) and spectral measurements. The inventory

and descriptions would be performed by the PET and be provided to CAPTEM and the science community at large to support decisions on sample sterilization and distribution.

6.4.1.4 Perform Selected Sample Analyses. While in principle we advocate minimizing the amount of analysis that is done in the SRF, some analyses will need to be done there because:

(1) They are necessary for choosing the samples to be sterilized and distributed to the science community.

(2) They are time critical.

(3) They cannot be done on sterilized samples and so must be done at the SRF or another containment facility.

Some analysis may be judged to be time critical because of public pressure to get early results on samples or because sample deterioration prevents the analyses being done at a later date. It will, for example, be desirable to do some life detection tests in the SRF almost immediately upon the sample receipt, because of public interest to get an early assessment of the possibility of life in the samples. Moreover, such preliminary tests will help design subsequent tests. Such preliminary tests are, however, unlikely to be definitive.

Life detection tests fall into two categories: those for detection of extant life and those for detection of past life. Much of the early chemical and morphological search for past life can be done on sterilized samples. Testing for viable organisms can only be done on unsterilized samples and must be done behind a biobarrier. This testing could be done either in the SRF or an equivalent facility that has the required safeguards for both protecting the samples and preventing uncontrolled release. Because of the rapid evolution of biological testing techniques, it is premature at this time to judge what techniques might be used to detect extant life in 2008 when the first samples will be returned.

Some analyses will need to be done at the SRF because they cannot be done at a later date. With lunar samples, for example, short-lived radionuclides were determined immediately upon receipt, before they could decay to undetectable levels. Because of the long transit time from Mars, there is less need for immediate analyses, although determination of labile constituents in the soil may be advisable since significant changes may occur under laboratory conditions.

6.4.1.5 Test for Possible Hazards. One of the prime functions of the sample receiving facility is to test the samples for possible hazards. The protocols for hazard testing will likely be developed over the next several years by the equivalent of Apollo's Biosciences and Containment Group under the direction of a committee equivalent to the ICBC. An indication of what that testing might be is given in the recommendations of the Biohazard and Testing Subgroup of the Mars Sample Quarantine workshop held at NASA/Ames in 1997.** The following is an abstract of the subgroup's recommendations.

(1) Chemical Toxicity. Because of the small amount of material to be returned, chemical toxicity is not considered a significant threat.

**Draft in review: DeVincenzi, D.L., et al. (editors), 1998. *Proceedings, Mars Sample Quarantine Protocol Workshop*, NASA/Ames, June 4–6, 1997, Preliminary draft. NASA/Ames internal document, 6/12/98.

(2) Pathogenicity. Pathogenesis can be of two types: toxic, in which metabolic products or cell components incidentally harm other organisms, and infectious in which the agent must multiply in or on the host to cause damage. Both should be tested for in the martian samples. The Apollo program challenge test protocols initially included 69 species from 10 animal phyla and 34 species in nine plant divisions. Consequently, it required elaborate animal support and contamination controls within the quarantine facility (Allton, 1998). In light of the significant advances in the use of model systems and tissue cultures, as well as improvements in technological capabilities over the past few decades, the subgroup concurred that it would not be necessary to conduct whole organism challenge tests as part of the first-line screening for returned martian samples. In-vitro methods are deemed far superior 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. The subgroup recommended in-vitro testing of the following, all of which could be done in Class III biosafety cabinets:

(a) Diverse microbial media using varied laboratory starting conditions

(b) Selected tissue cultures and cell lines from: mammalian organ systems, fishes, and insects

(c) Embryonating chicken eggs

(d) Plant tissue culture (wheat, rice, potato)

In addition the subgroup discussed inclusion of two types of whole organism 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). Such tests might be included both for science reasons and for reasons of public acceptance.

While it is considered very unlikely that putative martian organisms could compete successfully against terrestrial organisms in terrestrial habitats (see section 1.3 above), there are uncertainties. The Biohazard and Testing Subgroup at the June 4-6, 1997 (DeVincenzi et al.) Ames workshop accordingly recommended two types of microcosm tests. The first was designed to assay for disruptions of important representative microbial systems upon addition of martian materials, and the second was to determine if any martian biological entities can grow or propagate in selected microcosms of representative terrestrial ecosystems. Details are given in Appendix B of DeVincenzi, et al. (1999).***

What constitutes a representative sample will be an important component of the analytical strategy, and this may not be resolvable until more information is known about the samples actually returned.

***Draft in review: DeVincenzi, D.L., et al. (editors), 1998. *Proceedings, Mars Sample Quarantine Protocol Workshop*, NASA/Ames, June 4–6, 1997, Preliminary draft. NASA/Ames internal document, 6/12/98.

6.4.1.6 Sterilize a Subset of the Samples. **The SRF must have the capability of sterilizing a subset of the samples for release from the facility and distribution to the science community.** This implies capabilities within the SRF for making splits of individual rock and soil samples and for doing the sterilization. Based on the work of Allen (1998), we are recommending gamma radiation as the sterilizing agent. It is effective, yet minimizes changes to the samples. Commercial sterilizers are available. The viruses Ebola and Lassa are inactivated at < 1 Mrad, and the USDA recommends 6 Mrad for virus inactivation in biological materials. D. radiodurans, a radiation-resistant bacterium, is sterilized at 3–8 Mrad. The spore-forming bacteria B. subtilis and C. sporogenes in basalt cores have been sterilized in the 3–30 Mrad range. The doses should significantly exceed those required to deactivate any known form of microbial life. The dose levels are to be determined, but dosages in excess of 30 Mrad may be needed.

We are recommending that work on gamma sterilization be aggressively pursued to determine the gamma ray toleration limits of radiation-resistant terrestrial life forms and to better assess the effects of high radiation doses on different rock materials. The strategy for sample handling outlined in this chapter is totally dependent on our being able to distribute early sterilized samples to the science community. If this capability is not available, then far more analyses would need to be done in the containment facility, and the SRF would be correspondingly more complex and expensive.

6.4.2 Options to be Considered in Design of Sample Receiving Facility

6.4.2.1 Duty Cycle: An SRF Facility That Is Used Only Intermittently

(a) Design based on the assumption that hazard testing will prove the samples are harmless. In this case the SRF is used intermittently, for a short period after each sample-return mission, after which the samples are transferred to the SCF.

(b) Design assumes that the samples will be hazardous, that unsterilized samples will remain permanently in the SRF, and that any analyses requiring unsterilized samples will be done in the SRF or some other biocontainment facility.

6.4.2.2 Materials Examined: Mars Samples Only or Any Extraterrestrial Materials

(a) Facility is used exclusively for martian materials.

(b) Facility is used for all extraterrestrial materials that are judged to require containment and hazard assessment.

6.4.2.3 Level of Instrumentation Needed

(a) Analyses at the SRF are restricted to benchtop testing, and samples (both sterilized and unsterilized) are shipped to other facilities for analyses requiring more complex instrumentation.

(b) SRF is built to satisfy all the analytical needs that are anticipated for the time prior to their validation as harmless, after which the capabilities of the community at large could be utilized.

6.4.2.4 Example of a Processing Plan. The following processing plan (Table 6-1) envisions a relatively modest Mars SRF for the purpose of quarantining the samples and

Table 6-1. Example of a Mars Sample Processing Plan

PET Activities Inside Biobarrier	Outside Biobarrier
Receive sample container into Mars SRF Extract and analyze gases in head space Remove samples and document types: weigh, photograph, number, etc. Perform optical examination of samples Select sample suites for testing Store sample remainder in martian ambient	
Conduct basic biohazard/life tests (in-vitro?) Gamma sterilize some material for additional tests	Conduct preliminary analyses on sterilized sample for total carbon, organics, chemical composition, mineralogy, and TBD, in selected laboratories
If life/biohazard/organics detected	**If no life/biohazard/organics detected**
Proceed below	Decision for controlled release
Prepare quarantined sample for more extensive biohazard testing in state-of-the-art laboratories (whether inside or outside Mars SRF is TBD)	Detailed bio/organic analyses of witness plates Possible additional geochemical analyses on sterilized samples
If martian life/biohazard confirmed	**If no martian biohazard detected**
Perform all analyses inside quarantine or on sterilized samples	Decide about controlled release Transfer part/all of samples to curation facility Allocate for detailed scientific analyses worldwide

conducting preliminary geochemical and biohazard analyses. Additional preliminary geochemical analyses on sterilized samples could be conducted in outside laboratories. If any indications exist for complex organics or biohazards in the samples, the plan envisions prearranged elaborate biohazard testing of martian material in outside, variously instrumented laboratories as well as full analyses of witness plates taken before and during the mission. This plan would allow the most efficient use of limited mission resources under the most likely scenario that the probability of detection of a biohazard in returned martian samples is very small. The plan would also involve selected outside laboratories in sample analyses and testing early in the process. A different sample-processing plan would be to construct a large and complex containment facility in which a broad variety of biohazard, life detection and geochemical analyses could be conducted. Full justification for such a facility would exist only in the low probability case that a biohazard is found to be present in the martian samples.

6.5 Sample Curation Facility (SCF)

Once samples have been removed from biocontainment, responsibility for their custody shifts from the SRF to the SCF. Removal of samples from biocontainment may be enabled because they are sterilized or because the samples have been found to be harmless. Release of sterilized samples may take place within days or weeks of return of the samples. Determination that the samples are harmless could take months to years. In the unlikely event that the samples are found to be harmful, then the samples would have to be permanently stored under appropriate biocontainment within the SRF or elsewhere (or sterilized).

The main functions of the SCF are to preserve the samples and to make them available to the community for research and education. The existing curation facility at JSC currently performs these functions. It has 30 years experience in handling extraterrestrial materials, and we see no reason why it should not continue to perform the same functions for martian samples.

6.6 Management Issues

A dual management structure similar to that adopted by Apollo may be required, with one entity being responsible for addressing planetary protection issues and a second entity being responsible for science. The planetary protection management would be responsible for assessing whether the samples present any threat. The science management would be responsible for doing the scientific evaluation, including whether the samples contain evidence of past life or present life. The management structure must incorporate mechanisms for resolving routine scientific conflicts, such as might arise over sample allocation and facility use.

6.6.1 Planetary Protection Management

6.6.1.1 Interagency Committee on Back Contamination (ICBC). An international committee like the ICBC should be established to provide advice on planetary protection issues, such as certifying that the samples are safe for release and establishing protocols for testing for biohazards.

6.6.1.2 Planetary Protection Team. A Planetary Protection Team should be established to implement the recommendations of the ICBC. **The team should consist mostly of permanent staff that should be in place at the SRF 1–2 years prior to receipt of samples in order to develop procedures for assessing if the samples are hazardous, to test those procedures, and to become thoroughly familiar with protocols for working within the containment facility.** This permanent staff may be supplemented by a small number of non-resident scientists, but they must also expect to spend a significant fraction of their time in the year before sample receipt, at the SRF familiarizing themselves with the operation of the facility.

6.6.2 Science Management

A guiding principle behind the procedures for handling martian samples outlined here is to capitalize as much as possible on the analytical capabilities of the scientific community at large, and to minimize the amount of analysis done at the sample receiving facility. We envisage that most of the science will be managed in the same way that science on other extraterrestrial materials is managed, that is through the NRA and peer review process. A possible exception is the work done by the Preliminary Examination Team (PET). It will do the basic characterization of the samples needed to formulate the subsequent analytical plan. Analyses at the SRF should be limited to those required (1) to intelligently distribute sterilized samples, (2) to determine if the samples are harmful, or (3) because of their time criticality. Almost all the analyses recommended above to be done at the SRF are benchtop tests, not requiring elaborate instrumentation. We emphasize that the SRC should be a service facility, not a research facility.

6.6.3 PET

The initial characterization of the samples would be done by a Preliminary Examination Team (PET) under the guidance of CAPTEM or its equivalent. The functions of the PET would be to:

(1) Extract the samples from the sample container and package them appropriately.

(2) Do the preliminary characterization of the samples.

(3) Prepare sample splits for sterilization, hazard analysis, and other analyses deemed necessary.

(4) Sterilize a subset of the samples.

(5) Perform time critical analyses, as required.

Like the Planetary Protection Team, the PET should consist largely of permanent staff. It should be selected and in place at the SRF 1–2 years before receipt of the samples in order to develop procedures for handling the samples and to become thoroughly acquainted with those procedures. This permanent staff may be supplemented by a small number of non-resident scientists, but they must also expect to spend a significant fraction of their time in the year before sample receipt at the SRF familiarizing themselves with the operation of the facility.

6.6.4 CAPTEM

CAPTEM, or its equivalent, will advise NASA on the activities of the PET, on managing the longer-term curation of the samples, and on allocating them to the science community. Its functions would be to:

(1) Develop a long-term strategy for scientific analysis of the samples, including life-detection experiments.

(2) Provide oversight on the activities of the PET.

(3) Advise NASA on the content of the NRA for scientific analysis of released samples, conduct peer review of proposals, and advise on allocation of samples to chosen experimenters.

(4) Advise NASA on preservation and long-term curation of the samples at the SCF.

7 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 returned 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).

8 REFERENCES

Adler, M., Sept. 1998, *Planetary Protection Options*, (JPL internal document) JPL D-1675, Jet Propulsion Laboratory, Pasadena, CA.

Allen, C.C., 1999. Effects of sterilizing gamma radiation on Mars analog rocks and minerals. Abstract, *LPSC XXX.*

Biemann, K., et al., 1977. The search for organic substances and inorganic volatile compounds in the surface of Mars. *J. Geophys. Res.,* 82, 4641–4658.

Carr, M.H., 1996. *Water on Mars.* Oxford University Press, 229 p.

Carr, M.H., 1998. *Mars Sample Handling Requirements Panel (MSHARP) Meeting #6 Minutes* (JPL internal document) JPL D-16753, Jet Propulsion Laboratory, Pasadena, CA, December 14–15, 1998.

Carr, M.H., Nealson, K., Thompson, T., Sept. 1998. *Viewgraphs from the MSHARP Protection Meeting #5 held September 23–25, 1998* (JPL Internal Document), JPL D-16752, Jet Propulsion Laboratory, Pasadena, CA.

CDC–NIH, 1993, Biosafety in microbiological laboratories, 3rd Edition. Health and Human Services Publication No. (CDC) 93-8395, U.S. Govt. Printing Office, Washington, D.C.

DeVincenzi, D.L., and Bagby, J.R., 1981. *Orbiting quarantine facility: The Anteus Report.* NASA SP–454, 134 pp.

DeVincenzi, D.L., Stabekis, P. and Barrengoltz, J., 1996. *Refinement of planetary protection policy for Mars missions. Adv. Space Res.*, 18, (1/2)311–(1/2)316.

Fry, J.C., and Day, M.J. eds., 1992. *Release of genetically engineered and other micro-organisms*, Cambridge University Press, Cambridge, MA.

Gladman, B., 1997. Destination: Earth. Martian meteorite delivery. *Icarus*, 130, 228–246.

Gooding, J.L., 1990. *Scientific guidelines for preservation of samples collected from Mars.* NASA Technical Memorandum 4184.

Gooding, J.L., Carr, M.H., and McKay, C.P., 1989. The case for planetary sample-return missions. 2. History of Mars: *EOS, 70, 745,* 754–755.

Graf, P., 1971. *Lunar Receiving Laboratory Cleaning Procedures for Contamination Control,* NASA – TM-X-70162 (MSC-03243, JSC 03243) October.

Halliday, I., Blackwell, A.T., and Griffen, A.A., 1989. The flux of meteorites on the Earth's surface. *Meteoritics*, 24, 173–178.

Hochstein, L.I., Kvenolden, K.A., and Philpott, D.E., 1974. *The effect of sterilization on biological, organic, chemical, and morphological information in natural samples.* NASA-TM-X-72883, Ames Research Center, April 11, 74 pp.

Horneck, G., Bucker, H. and Reitz, G., 1991. Long term exposure of bacterial spores to space. NASA Conference Pub. 3134, Part 1 (A.S. Levine, ed.), 1667–1677. Washington, DC.

Jet Propulsion Laboratory, 1998. *Mars Sample Handling Requirements Panel (MSHARP) Meeting #2 Minutes of JPL, Pasadena* (JPL internal document) JPL D-16749, Jan. 9, 1998.

Jones, J.H., and Treiman, A.H., 1998. *Mars sample-return; Issues and opportunities.* Lunar and Planetary Institute, August 12, 1998.

Kaplan, D., 1988 *Environment of Mars,* 1988. NASA Tech. Memo. 100470.

Klein, H.P., 1978. The Viking biological experiments on Mars. *Icarus,* 34, 666–674.

Kuhn, W.R., and Atreya, S.K., 19779. Solar radiation incident on the martian surface. *J. Molecular Evolution,* 14, 57–64.

Labeda, D.P. et al., Soil Sterilization Effects on *in situ* Indigenous Microbial cells in Soil, *Can. J. Microbio* 21:263-269 (1975).

LDEF — 69 months in space. 1991 NASA Conference Publication 3134.

McKay, D.S., Gibson, E.K., Thomas–Keprta, K.L. Vall, H., Romanek, C.S., Clemett, S.J., Chillier, X.D.F., Maechling, C.R., and Zare, R.N., 1996. Search for past life on Mars: Possible relic biogenic activity in martian meteorite ALH84001. *Science,* 273, 924–930.

Mitchell, F.J., and Ellis, W.L., 1972. "Surveyor 3. Bacterium isolated from lunar–retrieved television camera." in, *Analysis of Surveyor 3 material and photographs returned by Apollo 12. NASA SP-284,* 1972.

NASA 1965 Summer Conference on Lunar Exploration and Science, Falmouth, Massachusetts, July 19–31, 1965, NASA SP-88.

National Research Council, 1992. *Biological contamination of Mars: issues and recommendations*, National Research Council, Washington, D.C., 115 pp.

National Research Council, 1997. *Mars sample-return: issues and recommendations*, National Research Council, Washington, D.C., 1997, 47 pp.

National Research Council, 1998. *Evaluating the biological potential in samples returned from planetary satellites and small Solar system bodies: framework for decision making,* Washington, D.C., 1998, 100 pp.

Squyres, S.W., 1996. Mars Surveyor Program '01 integrated payload proposal. Center for Radiophysics and Space Research, Cornell University, Ithaca, NY.

Townsend, J.E., 1990. *Mars Sample Receiving Facility.* NASA JSC Publication 24736, Johnson Space Center, Houston, TX 25 pp.

Treiman, A.H. and Jones, J.H., 1998. *Mars sample-return: issues and opportunities.* A CAPTEM report. Informal report, Lunar and Planetary Institute, Houston, 1998.

APPENDIX A

EFFECTS OF DRY HEAT TREATMENT ON BACTERIAL SURVIVAL (IMPLICATIONS FOR MARS SAMPLE RETURN)

presented to:

MSHARP Committee Meeting
December 14, 1998

by:

Donald L. DeVincenzi
NASA Ames Research Center

and:

Harold P. Klein, Sara E. Acevedo, Robert D. Howell
SETI Institute

EFFECTS OF DRY HEAT TREATMENT ON BACTERIAL SURVIVAL

- "... naturally occurring spore populations are composed of organisms having a wide range of heat resistance."
  Bond *et al.*, *Appl. Microbio* **21(5)**:832 (1971).

- "Heating intervals ranged from 1 to 12 hr at 125 °C and 6, 12, 18, and 24 hr at 113 °C. Eight hours was the longest heating time yielding survivors at 125 °C, whereas survivors were recovered at all the heating intervals at 113 °C."
  Puleo *et al.*, *Appl. Microbio* **30(5)**:786 (1975).

- "At all temperatures tested [120 – 180 °C] the resistant fraction consisted of ca. 1×10^5 spores out of 6.2×10^7 spores in each sample."
  Molin and Östlund, *Antonie van Leeuwenhoek* **41**:329 (1975).

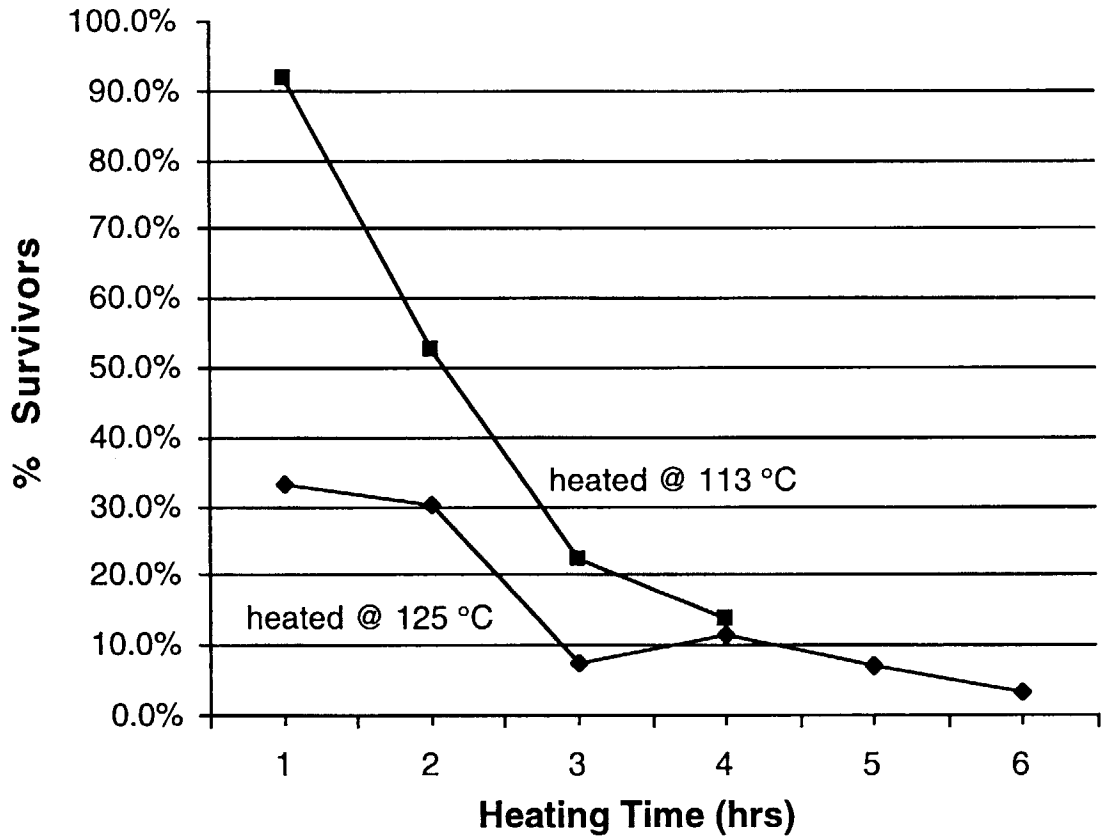
- "... naturally occurring bacterial spores in soil" were found to show "resistance to 125 °C (dry heat, D = 139 hr) ..."
  Bond and Favero, *Appl. Microbio* **29(6)**:859 (1975).

- "The dry-heat destruction rate of the naturally occurring spores at 105 °C was found to be extremely slow; the D value for the resistant sub-population was 101.54 hr."
  Sivinski *et al.*, "The Synergistic Inactivation of Biological Systems by Thermoradiation," in: *Industrial Sterilization*, G.B. Phillips and W.S. Miller, Eds., Duke University Press, Durham, NC (1973).

- "The dry heat treatment received by the surface of a potato during baking at 175 °C will not destroy all of the dry heat resistant spores on the surface."
  Ernst, "Sterilization by Heat," in: *Disinfection, Sterilization, and Preservation*, 2ed., S.S. Block, Ed., Lee and Febiger, Philadelphia (1977).

- "... spores occluded in crystal structures display a phenomenal resistance to destruction by heat." At 121 °C, spores of *B. subtilis var. niger* occluded in crystals of calcium carbonate gave an increased resistance in dry heat of 9-fold.
  Rank and Pflug, *J. Food Protection* **40**:608 (1977).

SURVIVAL AS A FUNCTION OF HEATING TIME



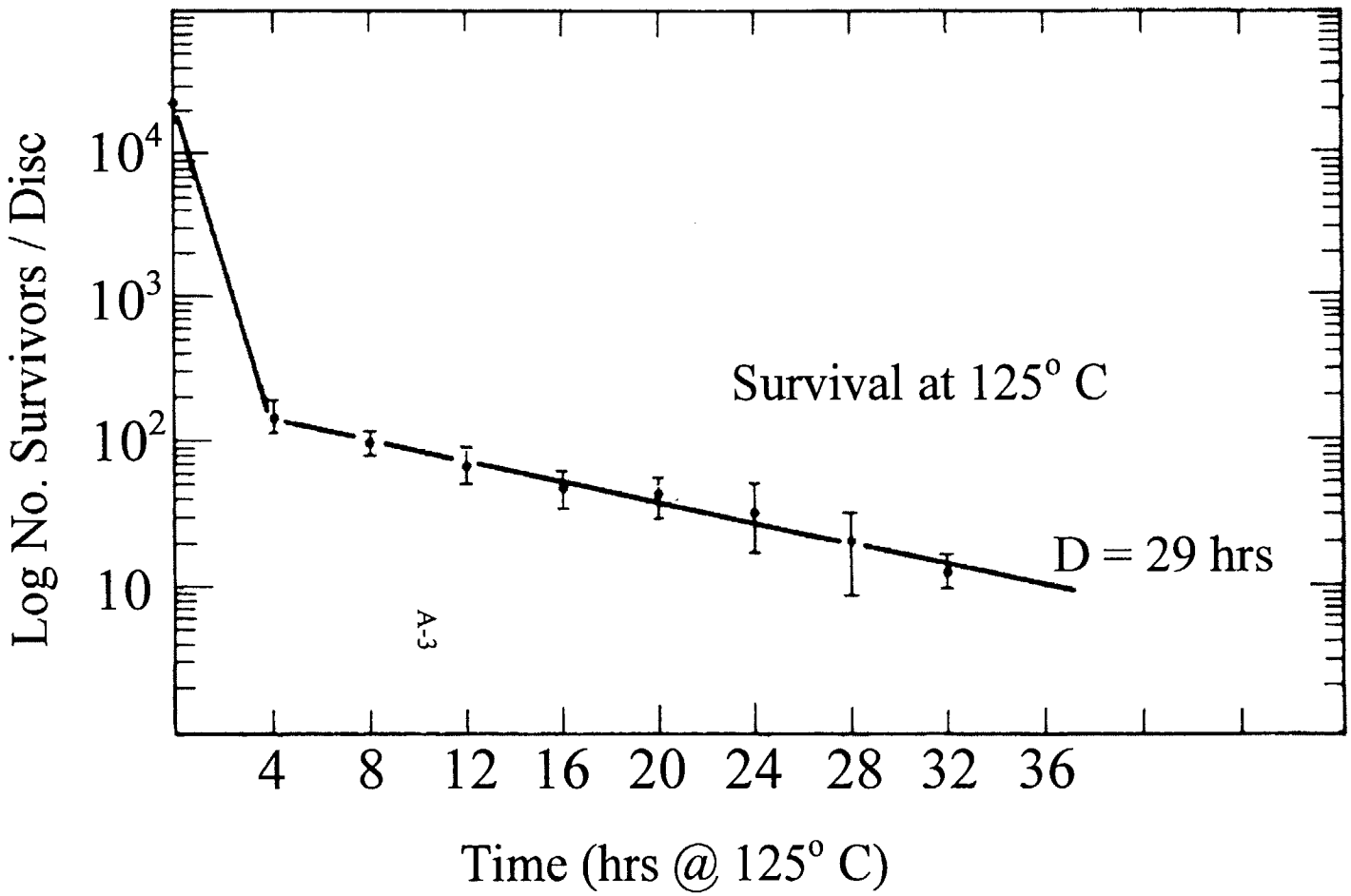
Adapted from: Puleo *et al.*, *Appl Microbio* **30(5):**786 (1975).
Results of Teflon Ribbon experiments with naturally occurring airborne spores.

EFFECT OF HEAT TREATMENT ON VIABILITY OF INDIGENOUS SOIL MICROORGANISMS

Treatment	Plate counts per gram soil after Treatment
Untreated	4.0×10^6
Dry heat 160 °C / 30 hr	3.0×10^2
Dry heat 200 °C / 24 hr	0
Autoclaving	0

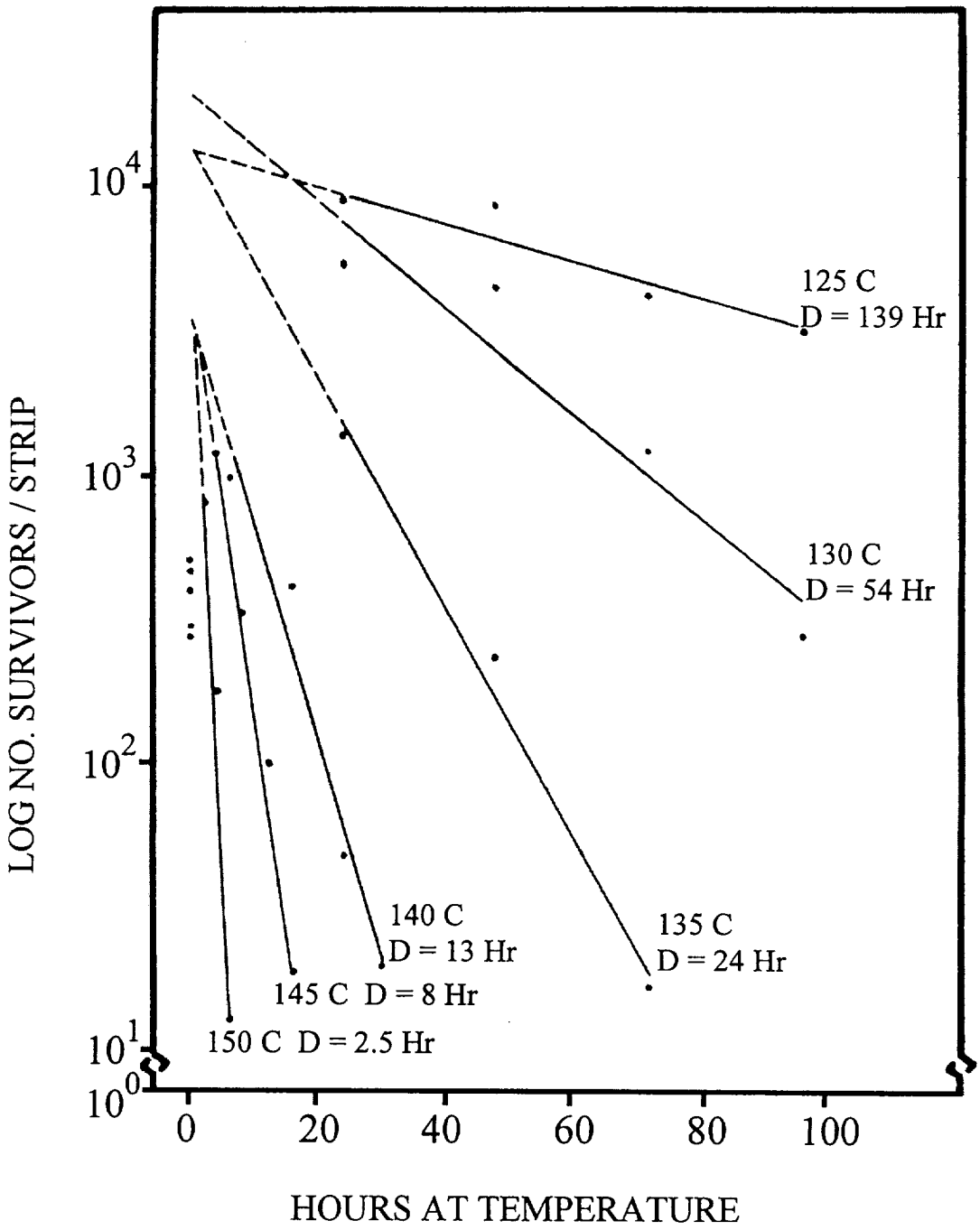
REF: Labeda *et al.*, *Can. J. Microbio* **21**:263 (1975).

DRY-HEAT INACTIVATION OF NATURALLY OCCURRING SPORES IN SOIL



REF: Reynolds *et al.*, *Appl Microbio* **28(3)**:406 (1974).

SURVIVAL OF *BACILLUS* SP. ATCC 27380 SPORES EXPOSED TO DRY HEAT



REF: Bond and Favero, *Appl. Microbio* **29(6)**:859 (1975).

EFFECTS OF DRY HEAT TREATMENT ON BIOLOGICAL COMPONENTS

- "The heat [160 °C, 3hr] proved to be detrimental to the structural integrity of most of the *in situ* cells in the soil as observed in thin sections ... the heat had caused too much structural disorganization for cell detection ..."

  Shih and Souza, *Origins of Life* **9**:51 (1978).

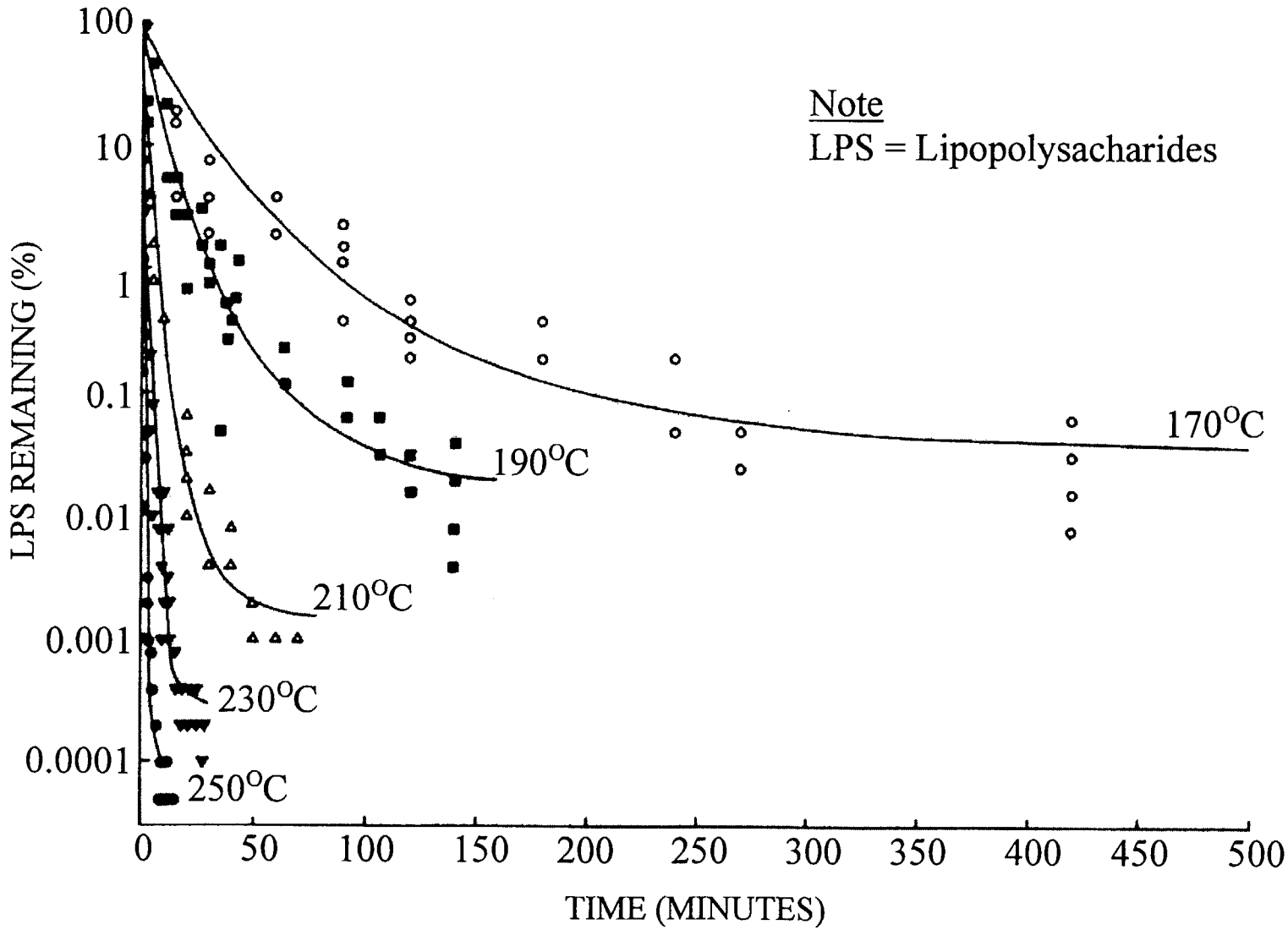
- "... loss of biochemical information occurs when soil is sterilized by dry heat. No enzymatic activity was detectable in soil sterilized [at] 148.5 °C for 4 days."

  Shih and Souza, *Origins of Life* **9**:51 (1978).

- Ionic interactions and the local environment of subunits play key roles in protein thermostability above 100°C.

  Vetriani *et al.*, *Proc. Nat. Acad. Sci. US* **95**:12300 (1978).

DRY-HEAT DESTRUCTION CURVES OF LPS FROM E. COLI



REF: Tsuji and Harrison, *Appl. Environ. Microbio.* **36(5)**:710 (1978).

SEM IMAGING OF THE EFFECT OF DRY HEAT TREAMENT ON BACTERIAL CELL INTEGRITY

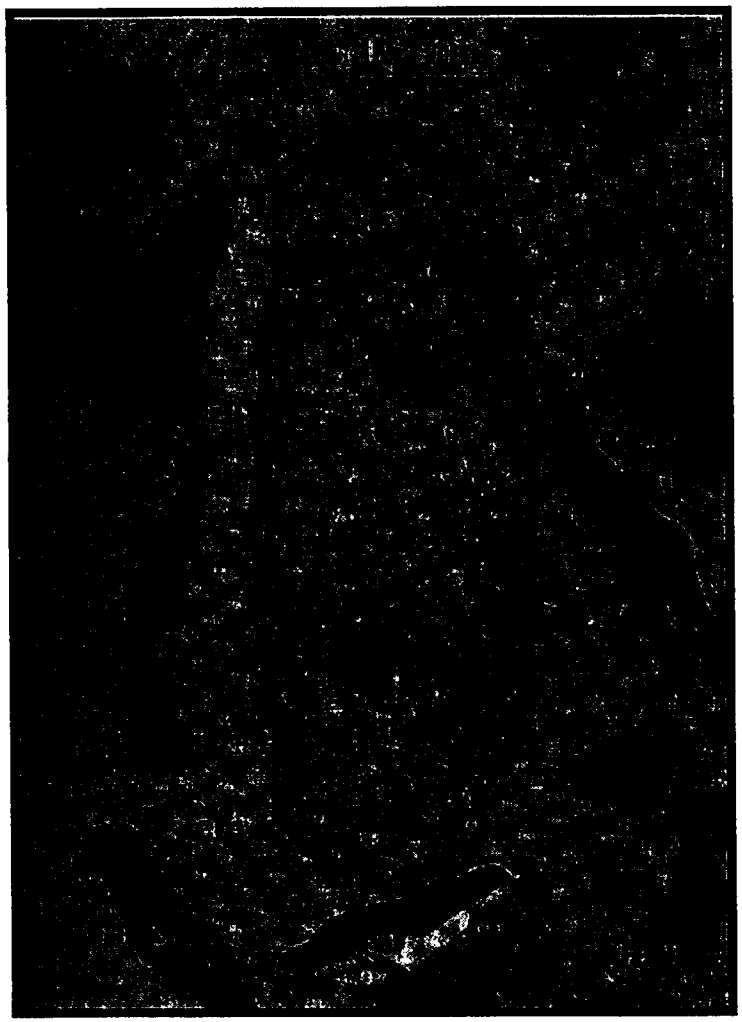


FIG. 1. Microbial cells from untreated (control) soil.

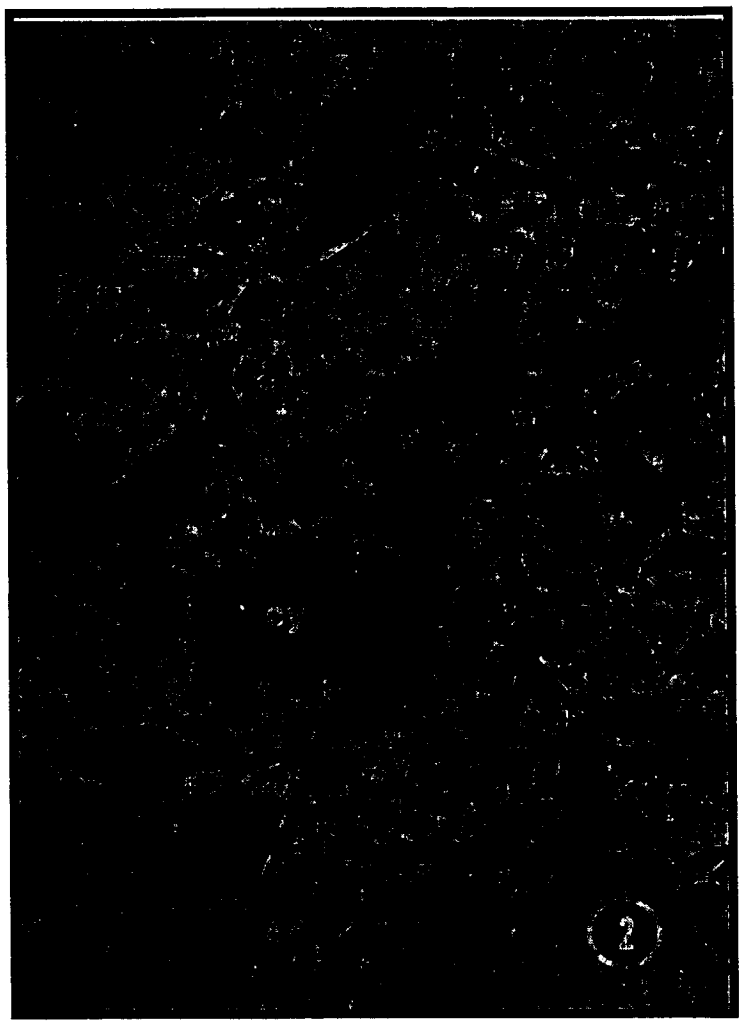


FIG. 2. Microbial cell from soil subjected to dry heat at 160C for 3 h.

REF: Labeda *et al.*, *Can. J. Microbio* **21**:263 (1975).

EFFECTS OF DRY HEAT TREATMENT ON GEOCHEMICAL COMPOSITION

- "... the trace quantities of oxidants decline in reactivity upon heating over the 47 – 127 °C range. Consequently, any hope of identifying the oxidants in returned martian samples would be lost if the samples were heated above ~47 °C."

  Gooding, J.L., Ed., *Scientific Guidelines for Preservation of Samples Collected from Mars*, NASA TM-4184 (1990).

- "Deliberate thermal sterilization of samples would irreversibly decompose heat-sensitive minerals, alter stable-isotope ratios, and possibly erase records of natural radiation doses that would be critical to paleoclimate studies."

  Gooding, J.L., Ed., *Scientific Guidelines for Preservation of Samples Collected from Mars*, NASA TM-4184 (1990).

- "Above the temperature generally recognized as adequate for biological sterilization (150 °C), many profound chemical and mineralogical changes would occur in samples. The most important would be ... loss of H_2O from hydrous silicates, oxides, and salts, and loss of CO_2 from carbonates."

  Gooding, J.L., Ed., *Scientific Guidelines for Preservation of Samples Collected from Mars*, NASA TM-4184 (1990).

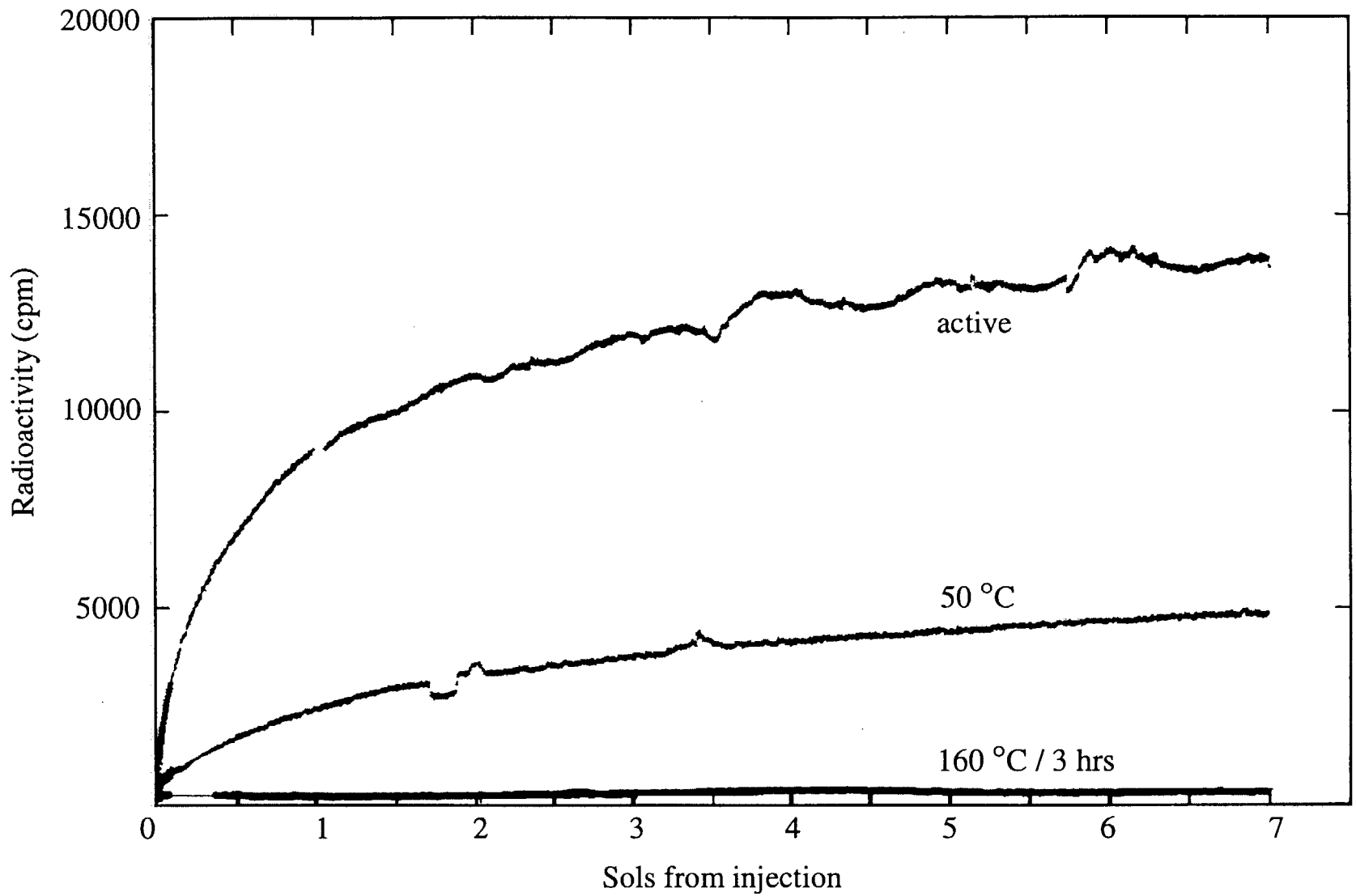
- "At temperatures >100 °C, oxygen isotope exchange reactions would occur at significant rates for clay minerals and related silicates."

  O'Neil *et al.*, "Preservation of H, C, and O Isotopic Ratios in the Low temperature Environment, in: *Stable isotope Geochemistry of Low Temperature Processes*," T.K. Kyser, Ed., Short Course Handbook, Vol 13 Mineralogical Society of Canada (1987).

- "... samples subjected to thermal sterilization might also suffer destruction of any fluid inclusions, resetting of mineral geothermometers, and annealing of radiation damage."

  Gooding, J.L., Ed., *Scientific Guidelines for Preservation of Samples Collected from Mars*, NASA TM-4184 (1990).

EFFECTS OF HEATING ON LABELLED RELEASE EXPERIMENT



Adapted from: Levin and Straat, *J. Mol. Evol.* **14**:167 (1979).

CONCLUSIONS

Dry-heat treatment of samples, even at moderate temperatures (~150 °C):

- is not likely to adequately 'sterilize' the sample
- will destroy some potential biological information present
- will destroy some mineralogic/geochemical information present

Even higher temperatures may be required in the absence of oxygen and water.

BIBLIOGRAPHY

Bond, W.W., "Relative Frequency Distribution of $D_{125\,C}$ Values for Spore Isolates from the Mariner-Mars 1969 Spacecraft," *et al., Appl. Microbio* **21(5)**:832-836 (1971).

Bond, W.W. and M.S. Favero, "Thermal Profile of a *Bacillus* Species (ATCC 27380) Extremely Resistant to Dry Heat," *Appl. Microbio* **29(6)**:859-860 (1975).

Ernst, R.R. "Sterilization by Heat," p. 481-522 in: *Disinfection, Sterilization, and Preservation*, 2ed., S.S. Block, Ed., Lee and Febiger, Philadelphia (1977).

Gooding; J.L., Ed., *Scientific Guidelines for Preservation of Samples Collected from Mars*, NASA TM-4184 (1990).

Labeda, D.P. *et al.*, "Soil Sterilization Effects on *in situ* Indigenous Microbial cells in Soil," *Can. J. Microbio* **21**:263-269 (1975).

Levin, G.V. and P.A. Straat, "Completion of the Viking Labeled Release Experiment on Mars," *J. Mol. Evol.* 14:167-183 (1979).

Molin, G. and K. Östlund, "Dry Heat Inactivation of *Bacillus subtilis* Spores by Means of Infra-Red Heating," *Antonie van Leeuwenhoek* **41**:329-335 (1975).

O'Neil, D. *et al.*, "Preservation of H, C, and O Isotopic Ratios in the Low Temperature Environment," in: *Stable isotope Geochemistry of Low Temperature Processes*, T.K. Kyser, Ed., Short Course Handbook, Vol 13 Mineralogical Society of Canada (1987).

Puleo, J.R. *et al.*, "Method for Collecting Naturally Occurring Airborne Bacterial Spores for Determining their Thermal Resistance," *Appl Microbio* **30(5)**:786-790 (1975).

Rank, E. and I.J. Pflug, "Dry Heat Destruction of Spores on Metal Surfaces and on Potatoes During Baking," *J. Food Protection* **40**:608-613 (1977).

Reynolds, M.C. *et al.*, "Thermoradiation Inactivation of Naturally Occurring Bacterial Spores in Soil," *Appl Microbio* **28(3)**:406-410 (1974).

Shih, K.L. and K.A. Souza, "Degradation of Biochemical Activity in Soil Sterilized by Dry Heat and Gamma Radiation," *Origins of Life* **9**:51-63 (1978).

Sivinski, H.D. *et al.*, "The Synergistic Inactivation of Biological Systems by Thermoradiation," p. 305-335 in: *Industrial Sterilization*, G.B. Phillips and W.S. Miller, Editors, Duke University Press, Durham, NC (1973).

Tsuji, K. and S.J. Harrison, "Dry Heat Destruction of Lipopolysaccharide: Dry-Heat Destruction Kinetics," *Appl. Environ. Microbio.* **36(5)**:710-714 (1978).

Vetriani, C. *et al.*, "Protein Thermostability Above 100 °C: A Key Role for Ionic Interactions," *Proc. Nat. Acad. Sci. US* **95**:12300-12305 (1978).

Appendix B
CAPTEM Inputs on Sample Return

B-3 CAPTEM Reiew of Moecular Tracer Concept

B-9 Issues Raised by Steve Squyres Concerning Collection of Martian Samples

Institute of Meteoritics 1944
UNIVERSITY OF NEW MEXICO

Dr. James J. Papike
Director and Regents' Professor
Institute of Meteoritics
Earth & Planetary Sciences
University of New Mexico
Albuquerque, New Mexico 87131
Telephone: (505) 277-1644
FAX: (505) 277-3577
E-Mail: jpapike@unm.edu

MEMORANDUM

To: Michael H. Carr, Chair, MSHARP

From: J.J. Papike, Chair, CAPTEM

Subject: CAPTEM Review of Molecular Tracer Concept For Mars 2001 Mission

Date: March 31, 1998

At the request of Dr. Steve Squyres, CAPTEM conducted a review of the potential use of molecular tracers for detection of organic/biologic contamination of Martian samples to be collected by the Athena payload on the Mars 2001 mission. The CAPTEM review procedure and recommendations are attached. This report is being formally submitted to MSHARP which is the appropriate committee to be concerned with such issues.

MSHARP will have to decide on how to deal with these recommendations. If MSHARP agrees with the report, then the recommendations can be passed on to Dr. Carl Pilcher and other appropriate NASA administrators.

xc: MSHARP Committee Members
S. Squyres
S. Saunders

LUNAR AND PLANETARY INSTITUTE
3600 BAY AREA BOULEVARD HOUSTON, TEXAS 77058-1113
TEL (713) 486-2139 FAX (713) 486-2162

March 27, 1998

A CAPTEM* Assessment of the Use of Molecular Tracers for Detection of Organic/Biologic Contamination of Martian Samples to Be Collected by the Athena Payload on the Mars 2001 Mission Rover.

BACKGROUND

On January 9, 1998 the Athena team for the Mars 2001 Mission gave a briefing to MSHARP (Dr. M. Carr, Chair) on a molecular tracer technique that was proposed to be used to evaluate the degree to which returned martian samples had been contaminated with terrestrial biological/organic materials. Such an evaluation is important because, during the analysis of returned samples, round-trip terrestrial contamination (i.e., materials that traveled with the Athena and Rover to Mars and returned back to Earth) might be mis-identified as martian organic material or even as viable martian organisms. As a result of the briefing, MSHARP expressed concern to the Athena PI, Dr. S. Squyres, about using the tracer technique but also recommended that he get further input from a knowledgeable panel of sample scientists. Dr. Squyres then requested that CAPTEM review the tracer technique and its probable impact on the sample return mission.

Consequently, on March 21, 1998, CAPTEM convened a special session at the Lunar and Planetary Institute (Houston), and heard presentations by Dr. S. Squyres (Athena team PI) and Dr. T. Onstott (proposer of the molecular tracer technique). The committee was composed both of regular CAPTEM members and of a *pro tempore* membership, invited in order to broaden CAPTEM's scientific expertise (membership of the molecular tracer review subcommittee is given below). CAPTEM was briefed about the Athena and Rover Mission as well as about the molecular tracer technique. Mars samples are to be collected by a coring tool on the Athena rover. The contamination assessment scheme involves intentional introduction of molecular tracer materials into the coring drill assembly (and onto the Rover in general). The tracer materials could then be analyzed in the drill core samples (after return to Earth) as monitors of Earth-originated contamination that had entered the samples during drilling, handling, storage, and processing.

Interest in molecular tracers of terrestrial contamination comes from concerns that spacecraft surfaces may harbor viable micro-organisms (despite efforts at sterilization), that spacecraft surfaces may not be free from molecular residue of microorganisms that were killed during sterilization, and that non-sterile non-organically clean surfaces (like Rover wheels) may inadvertently contact samples designated for return. Round-trip transport of viable

* CAPTEM is the Curation and Analysis Planning Team for Extraterrestrial Materials, convened by the NASA Discipline Scientist for Cosmochemistry, Joe Boyce.

microorganisms during a Mars mission appears to be a reasonable possibility. For example, viable bacterial spores apparently survived two and a half years exposure to the lunar environment on the Surveyor 3 lander, before being returned to Earth by the Apollo 12 mission. In a controlled experiment, large numbers of *Bacillus* spores survived exposure to orbital conditions (with minimal shielding) for six years on the LDEF satellite. Similar survival rates could be expected for microbial stowaways on Mars missions. Viability aside, methods used to examine Martian samples will undoubtedly detect biomolecules, such as nucleic acids or proteins, that could remain even after microorganisms are effectively killed. While the best possible solution would be to avoid transporting outbound microorganisms, practical considerations suggest that some outbound bio-contaminant will be inevitable.

To monitor and assess these potential sources of contamination in returned samples, the Athena team considered the application of easily detected, easily analyzed molecular tracers to Athena and Rover components. The tracer concept offers an attractive, relatively low cost means of detecting contamination in a sample, under appropriate circumstances. Information from tracers can potentially be both qualitative – indicating that communication between a sample and an external environment has occurred – and quantitative. If proper mass balance measurements are made, the degree to which a particular signal can be attributed to contamination can be estimated. However, the sources of potential contamination must be clearly defined and at least partially characterized for tracer methods to work. Viable tracers must be added directly to the source of contamination and must move into the sample in the same way that the contaminants do. Where multiple types and sources of contamination are possible, multiple tracers must also be used. When in the sample, they must be distinct from native materials, and easily detected – preferably with a detection limit several orders of magnitude lower than the contaminants of interest.

As presented to CAPTEM, molecular tracer schemes have been used successfully in searches for microorganisms deep in the Earth's crust. These organisms can be retrieved only by drilling, but the drilling environment permits extensive contamination of retrieved cores. The most important contaminant carrier is drilling mud, which is used to lubricate, pressurize, and sometimes power the drilling operation. Drilling mud can be forced into the drill core and into the rock surrounding the core, both of which could be sampled for indigenous microorganisms. Molecular tracer compounds added to the mud allow rapid quantification of the level of mud contamination, and permit the analyst to know (in a statistical sense) whether the organic and biological load in a particular sample could have come entirely from the drilling mud. Specific tracers mentioned include fluorinated hydrocarbons (e.g., perfluoromethlycyclohexane), and latex beads laced with fluorescent dyes.

RECOMMENDATION BY CAPTEM:

A subcommittee consisting of regular CAPTEM members and *pro tempore* members selected for their expertises (listed below) considered the proposed scheme of deploying molecular tracer materials, as well as an alternative scheme of monitoring terrestrial contamination using witness plates. The subcommittee was impressed with the Athena team's efforts to understand and control round-trip terrestrial contamination, and agreed that efforts to understand, monitor, and mitigate such contamination should begin now. However, the subcommittee found that intentionally introducing tracer contaminants onto the Athena package and the Rover would not

be advisable as described. Some details of our concerns are described below. There was much more support for the concept of using witness plates to monitor contamination end-to-end: before launch, in space, on Mars, and after return to Earth.

In general, the subcommittee was uncomfortable with the idea of intentionally deploying contaminant materials onto the Athena samples because of their possible effects on later analyses. The greatest concern was with tracer materials that contained the elements H, C, N, and O, which might have significant deleterious effects on Earth-based analyses focused on the biotic, pre-biotic, and climate history of Mars. Isotopically labeled tracer molecules could also lend undesirable complications to isotopic analyses. In addition, it was suggested that fluorescent tracer materials (e.g., latex spheres) might complicate subsequent biochemical analyses that use fluorescent markers. The subcommittee also explored the concept of using bio-mimics, like human-created, artificial DNA or RNA sequences in protein coats, as tracers; but it was felt that these particulates could needlessly complicate sensitive analyses for ultra-trace biologic molecules and structures. It was also noted that intentional addition of tracer molecules would require that each returned sample be analyzed for those molecules, probably at very low concentration levels. Such analyses could require significant sample mass (leaving less for other studies), or in the case of non-destructive analyses, further contaminate the sample being analyzed.

One suggested implementation of the molecular tracer technique would be for tracers to be introduced into the drill core assembly to monitor contamination during drilling. The subcommittee felt that intentional contamination during drilling was not necessary, as the coring process itself would likely add detectable chemical tracers to the samples. Materials from the drill bit and core sleeve are likely to be abraded during drilling, be rubbed onto rock core surfaces, and mixed into drill cuttings (dust). Analysis of core sleeve material rubbed onto the rock core surfaces could be used to evaluate surface contamination. Also, drill cuttings will enter the rock core along fractures and pores. So analysis of returned cores for traces of abraded drill could be used to evaluate particulate transport. For example, a type of tungsten carbide (WC) commonly used in drill bits contains 6% cobalt and detectable levels of tantalum. If drill cuttings (including bit material) have entered a drill core sample, their presence will be readily known by elevated abundances of cobalt (detectable at very low concentrations by neutron activation analysis), and could be confirmed by elevated abundances of tungsten and tantalum. Use of drill material as an "inherent tracer" will indicate whether the interior of each drill core communicated with the exterior during the mission; like the proposed molecular tracer scheme, it will not provide direct evidence of terrestrial contamination.

The subcommittee did not feel that an analogy between the contamination during terrestrial drilling and the contamination anticipated during the 2001 mission was completely appropriate. Unlike terrestrial drilling, potential sources and processes of terrestrial contamination during Mars mission operations are not understood well and the subcommittee suspected that some potential routes of contamination could bypass those of tracer delivery. If all sources of contamination cannot be traced effectively, then tracers could potentially be more confounding than helpful. For example, consider a scenario in which a molecular tracer is applied to non-sterile rover components, but that the largest potential source of biological contamination is airborne dust during Athena/Rover integration. These particulates are likely to be redistributed during launch,

and during the zero-gravity cruise to Mars, and could possibly migrate then to the return sample container and to the returned sample, independent of any tracer movement. The outcome of this scenario would be a return sample with viable (terrestrial) microorganisms but no molecular tracer. Logic would indicate, falsely, that the organisms originated on Mars. In this scenario, an effective tracer would have to permeate the environment during integration in approximately the same way as ambient microorganisms. The Athena payload instruments and the rover components will each experience a sequence of potentially contaminating environments before reaching Mars. An effective tracer scheme would require that every potential source of contamination in each environment be understood, and that each be dosed with an appropriate tracer. Unless sterilization and containment protocols can limit potential contamination to a few well-defined pathways, an effective tracer implementation seems problematic.

On the other hand, the subcommittee felt that witness plates had the potential to monitor all types of contamination, including round-trip terrestrial contamination, without an *a priori* understanding of all possible contamination processes. The subcommittee noted that the Athena rover would inevitably carry some Earthly materials (organic, biologic, and anthropogenic), which could themselves be analyzed as direct measures of contamination. The subcommittee further noted that data from witness plates or similar information were required as boundary conditions in order to design and quantify the molecular tracer technique. Thus, the sub-committee recommends that potential sources and processes of contamination be monitored through a series of witness plates. Some plates would be exposed to the same environment and potential contaminants as the Athena and Rover hardware on Earth until as late as possible before launch. Another witness plate would be exposed during the cruise phase to Mars, and closed on arrival. Another witness plate would be on the Athena package or Rover before and during Mars during operations to monitor terrestrial material that might enter the returned samples on Mars. All these witness plates would be returned to Earth for analysis and evaluation of potential round-trip terrestrial contamination.

The witness plate method for detecting contamination also has limitations. To be effective, witness plates would need to be exposed to *all* the same environments and conditions as the Athena payload and rover, and some would need to experience all sources of potential contamination *except* the Martian surface and samples. Witness plates, as a group, would be of limited effectiveness if significant "links" in this chain are broken, or if all were directly exposed to the Martian environment. In addition, witness plates will not provide a positive signal of contamination, but only a potential source for contaminant matching. For example, if a viable microorganism should be detected in a sample, it seems likely that release from quarantine would require that *the very same* kind of organism could also be detected on a protected witness coupon. At low contamination rates, with a diverse source community of microorganisms, this is unlikely. Conversely, if contamination rates are high, it might prove technically impossible to match all the detected organisms before the coupon sample is consumed. Thus, again, some idea of the likely contaminant load and the methods to be used for analysis must be determined before an effective strategy can be formulated.

The subcommittee was aware that the use of witness plates has cost and mass consequences for the mission. The use of witness plates on Earth, both before launch and after sample return, would

probably require relatively modest expenditures. Sample holder surfaces could act as witness plates for contamination summed over the whole sample return mission, and thus incur little cost or effort. But a witness plate that would be closed after the cruise phase to Mars, a witness to "out-bound" terrestrial contaminants, might have significant cost and mass consequences. A mechanism would be needed to close the plate upon arrival at Mars. And this witness plate would have to be on the return sample container, so it could be retrieved and returned to Earth for analysis.

SUMMARY

Although CAPTEM applauds the efforts of the Athena team to evaluate terrestrial contamination of returned Mars samples, CAPTEM views the molecular tracer technique (as presented) as flawed. With limited understanding of the processes by which the returned samples might be contaminated with round-trip terrestrial materials, a molecular tracer program cannot be properly designed. Dr. Squyres and Dr. Onstott indicated that some testing of potential contamination routes is proposed. These activities should be undertaken as soon as possible, to guide contamination control efforts. These exploratory experiments should include exposing control materials to all anticipated environments through which the Mars lander will pass, including launch pad, and if possible, launch conditions. Any proposed method for detecting contamination should be tested in this way, as soon as possible.

CAPTEM Molecular Tracer Subcommittee:

Member	Area of Expertise	Affiliation
J. Allton	Clean room techniques	Lockheed-Martin
Dr. D. Bogard	Noble gas geochemistry	NASA/JSC
Dr. R. Clayton	Stable isotope geochemistry	U. Chicago
Dr. J. Jones (chair)	Experimental geochemistry	NASA/JSC
Dr. L. Leshin	Ion microprobe analysis	UCLA
Dr. D. Lindstrom	Analytical geochemistry	NASA/JSC
Dr. J. Moldowan	Organic geochemistry/biomarkers	Stanford U.
Dr. T. Stevens	Biology in extreme environments	Pacific Northwest Lab.
Dr. A. Treiman (vice-chair)	Igneous/metamorphic petrology	Lunar & Planetary Inst.

Dr. J.J. Papike - Chair, CAPTEM

Dr. A.H. Treiman - Vice-Chair, Molecular Tracer Subcommittee (CAPTEM)

Dr. J.H. Jones - Chair, Molecular Tracer Subcommittee (CAPTEM)

Institute of Meteoritics 1944
UNIVERSITY OF NEW MEXICO

Dr. James J. Papike
Director and Regents' Professor
Institute of Meteoritics
Earth & Planetary Sciences
University of New Mexico
Albuquerque, New Mexico 87131
Telephone: (505) 277-1644
FAX: (505) 277-3577
E-Mail: jpapike@unm.edu

MEMORANDUM

To: Michael H. Carr, Chair, MSHARP

From: J.J. Papike, Chair, CAPTEM

Subject: CAPTEM Review of Issues Raised by Steve Squyres Concerning Collection of Martian Samples

Date: April 9, 1998

In addition to the CAPTEM review of the molecular tracer concept (earlier mailing to MSHARP), Steve Squyres asked for input on several other sampling issues described in the attachment. CAPTEM formally passes these recommendations to MSHARP for further consideration and action.

xc: MSHARP Committee Members
S. Squyres
S. Saunders

3-21-98 Sample Strategy Meeting

Athena 2001 Sample Collection and Storage: Potential Problems and Solutions

Report Prepared by CAPTEM

This report is presented in a series of bullets so the impact of the conclusions is not lost in wasted prose. Each point discussed is divided into the points raised for debate and the consensus reached by the sub-committee.

Sub-Committee Members:

C. Allen, Johnson Space Center
D. Des Marais - NASA/AMES
B. Jolliff - Washington University, St Louis
C.R. Neal - University of Notre Dame
J. Papike - University of New Mexico
G. Ryder, Lunar & Planetary Institute

OVERVIEW

The return of martian rock samples in pristine condition is vital for answering many scientific questions regarding the evolution of Mars and the possibility of life having existed on this planet. The sub-committee of CAPTEM, at the request of Steve Squyres, met to discuss five subjects related to the type, collection, and storage of samples by the 2001 Athena Mission:

1. Core Diameter
2. Sample-Sample Contact
3. Trace-Element Contamination
4. Soil Sample/Rock Sample Ratio
5. Seal Materials/Use of Teflon.

The current sample container contains 60 sample slots 5 cm deep which, if all are filled, means 300-350 grams of sample would be collected. **NOTE**: the corer has a maximum depth penetration of 5 cm but extracts this in two 2.5 cm samples.

1. Core Diameter.

Debate: if the diameter of the cores was smaller that 7 mm, drilling would be faster which would allow better sampling of a single rock.

Assuming 3 g/cm^3 density, a 5 cm core of 7 mm diameter yields 5.8 g. A 5 mm core reduces this mass by a factor of 2.

A smaller diameter core radically increases the contamination effects from the drill bit.

The larger diameter core allows for better sampling of unconsolidated soil material - with the new sample container depth (5 cm), soil samples could be collected down to 3-4 cm.

CONSENSUS: 7 mm diameter is the best diameter for the corer in order to collected the best samples while maintaining efficient drilling and core recovery.

2. SAMPLE-SAMPLE CONTACT.

DEBATE: The maximum length of any single core is 2.5 cm. In order to best use the sample container, two cores need to be put in the same sample slot.

Questions:

(a) should samples from different rocks be put in the same slot one on top of the other?

(b) Should cores from the same rock be put on top of one another as the deepest (freshest) surface of the second core would then be in contact with weathered outer surface of the first core?

The sample container could conceivably be 6 cm in depth, so a gap could be left between cores. However, these could move together during rover motions, lift off and impact.

Sample cross contamination is a big issue and experiments centered on drilling of different rock types by the corer without cleaning of the drill bit between samples are required (with subsequent geochemical analyses) prior to launch.

If only one 2.5 cm long core is put in each sample slot, only half the mass could be returned - a significant portion of which could be used up in "Planetary

Contamination Experiments" upon return as the mission plans to look at diverse rocks types, each of which would have to be tested for biological diversity.

CONSENSUS: It is most desirable to bring back the largest sample mass as possible, without compromising these samples by cross contamination.

Therefore, the recommended standard procedure should be that each rock selected for sampling have two cores extracted from the same hole and placed in the same sample slot (it is NOT desirable to mix lithologies within the same sample container unless absolutely necessary). This approach will mean that between every other core, no travel time is necessary so drilling/sampling time (dependent on the life of the batteries) is maximized along with sample return (through optimal use of the sample container). In addition, this approach dramatically reduces the risk of sample cross-contamination.

The surfaces of each core will be examined by the instrument array prior to storing in order to evaluate any contact between cores that may have occurred during the trip back to Earth.

The second 2.5 cm core will be less contaminated with the rock sample previously cored (or from the wire brush used to clean the bit between samples) as this would be removed during the first 2.5 cm core. REMEMBER: as the first core goes down the sample slot, it leaves dust - therefore it is essential to have the same lithology placed on top of this.

As there will be two cores from the same rock for almost all samples collected, it may be feasible to put half of the core on reserve for future analysis.

The first sample drilled will only be 2.5 cm - the first sample holder will be half full - this way the weathered surface remains relatively undisturbed and sample cross contamination is avoided (this is the first sample).

3. CONSOLIDATED ROCK SAMPLE/UNCONSOLIDATED SOIL SAMPLE RATIO.

DEBATE: Sixty (60) sample slots 7 mm diameter and 5 cm long are available. The present ratio is 53:7. This ratio depends on:

a) time required for rock coring;
b) time required for soil sampling;
c) diversity of soils - more area will be traversed than in Viking or even pathfinder, so greater soil diversity could be encountered.

NOTE: the terms "soil" is used here to mean unconsolidated/friable material only and represents sand & silt size fractions.

REMEMBER: the 2005 mission will collect a contingency soil sample.

If the soil is reasonably compact, a sample could be taken with the drill bit.

CONSENSUS: The ratio of rocks to soils should be 50*:10 so that soil diversity issues can be addressed. This committee recommends that attempts be made to collect two wind blown dust samples (Martian "loess") so crustal average compositions can be defined - this will be material ≤ 100 μm as this is the size range that can be moved by the martian atmosphere.

* the actual number of available slots for cores will be 49 because a pure, sterilized earth material (quartzite?) will be taken to and brought back from Mars in order to assess potential contamination (organic & inorganic) picked up during the mission.

4. TRACE ELEMENT CONTAMINATION.

DEBATE: Assuming the cached samples will be sealed prior to lift off and the sample container will not be breached upon impact, sources of contamination of the Mars samples will be:

a) Drill Bit and Core Housing;
b) Push Rod;
c) "Sheath" at the front end of the analytical instruments;
d) Wire Brush for drill bit cleaning;
e) Non-return "door" on sample slot to prevent sample from falling out (but see 5.).

While the drill bit design is not finalized, it is accepted that materials which offer the best drilling performance (cm per watt hour) should be chosen. This will probably be tungsten carbide coated with silicon nitride.

Tungsten carbide contamination of a rock sample seriously affects the analysis of W, C, Platinum Group Elements, Co, Zr, Nb, Hf, Ta and also W-Hf, Lu-Hf, + Re-Os isotope determinations.

The wear on the drill bit will be assessed on the surface through force/torque required to drill, current sensing(cm per watt hour), and visual inspection of the drill bit.

Inclusion of an enriched isotope tracer in the manufacture of the drill bit was discussed, and Steve Squyres will look into this, but offered no guarantees as to the inclusion of such material.

CONSENSUS: It is understood that mission design requires that drill bits be manufactured that are durable and efficiently produce the best cores. Drill bit and core housing contamination needs to be understood.

- Experiments on rocks of different lithologies and permeabilities# are required;
- The drill bit used in these experiments should be analyzed@;
- When the final drill bits, push rod, sheaths, wire brush, sample slot doors are manufactured, spares should be made at the same time so their composition can be determined@ as well as any elemental zonation@ in these materials.

Rocks of different permeabilities need to be drilled in order assess contaminant penetration into the interior of the core.

@ As many elements as possible should be quantified for these materials and by different analytical methods.

5. SEAL MATERIALS/TEFLON.

DEBATE: Although the sample container is under design, but each sample slot could have an pleated Teflon sleeve which would allow for slight expansion when the core is inserted, thus ensuring a tight fit of sample in the tube. Each sample tube could be surrounded by a deformable material to absorb vibrations and impact.

The end of each sample slot could have an iris-like door to prevent the sample from falling out, especially inportant for friable soil samples if they are gathered using the drill bit. However, the sample container may not be sealed.

PROBLEM: Static build up on the teflon could cause dust build up around each individual sample slot (and therefore potential contamination of the sample)

Sample holder design is ongoing - planetary contamination requirements are unclear and are being defined by MSHARP.

May seal the sample holder as one unit after all samples have been collected and stored, rather than each sample slot having a sealed door (cost). It is desirable to keep as much dust out of the sample container slots as possible.

but the samples need to tightly fit in the slots in order to prevent them "rattling around" during rover movement, lift off, and impact back on the earth. Deformable material to be placed around the sample slots to absorb impacts.

CONSENSUS: Teflon is good! All materials which will surround the sample should be made of Teflon, including the seals and sample slot doors. However, make sure that pure Teflon is used - this may require assaying of compnent material.

If the sample container itself is to be made of an aluminum alloy, this material also needs to assayed to define the elements present. Guidelines need to be defined on what Al-alloys are accaptable.

Symposium -- Rational Basis for Biocontainment

The Centers for Disease Control and Prevention (CDC) and the American Biological Safety Association (ABSA) sponsored a symposium titled "Rational Basis for Biocontainment" in Atlanta, GA January 17-20. ABSA is the professional society devoted to safe design and operation in microbiology laboratories. The mission of the symposium was "to provide an interactive forum for evaluating strategies for modern laboratory design and operation; to stimulate prospective thinking and proactive dialogue for finding new solutions for containment; and to publish a compendium of presentations and discussions that will serve as a valuable resource for those embarking on laboratory renovations or new construction." The symposium was hosted by Dr. Jonathan Richmond, Director of the Office of Health and Safety for CDC. Dr. Richmond has served as an advisor to NASA concerning biosafety issues of Mars sample return.

The symposium was particularly valuable in three areas related to Mars samples: communication between NASA and the biosafety community, sterilization of processing cabinets and geologic samples, and alternatives to containment laboratory construction.

- Astromaterials Presentation Margaret Race and Dr. Manuel Barbieto (Biological Safety Officer, US Department of Agriculture) presented a tutorial on the biosafety issues of the Apollo program and Mars sample return. Several audience members had participated in the design and operation of the Lunar Receiving Laboratory but this was the first presentation of Mars sample plans and concerns to the biosafety community.

- Sterilization of processing cabinets and HEPA filters Current planning to upgrade the JSC Meteorite Processing Laboratory, as well as planning for Mars sample laboratories, includes the ability to sterilize and decontaminate processing cabinets. The most common sterilization techniques in microbiological laboratories involve the use of gases (formaldehyde or ethylene oxide) and organic liquids. These techniques kill the organisms but inevitably leave organic residues. This is a serious concern for Mars meteorites and returned samples, which are and will be examined for trace levels of organics.

  An alternative that seems worth investigating is the use of vaporized hydrogen peroxide (VHP). This technique has been shown effective in killing bacterial spores and viruses, while leaving a minimum of organic residue. Research underway at the Baker Company, a major supplier of biological safety cabinets, is defining the equipment and operating parameters appropriate to VHP sterilization of biological safety cabinets and HEPA filters. Note that a more aggressive version, utilizing hydrogen peroxide plasma, is planned for sterilizing portions of the 2001 lander.

Appendix C. Acronyms and Unusual Terms

A5	Ariane 5
ALH84001	martian Antarctica meteorite with possible signs of life
APXS	alpha proton X-ray spectrometer
Athena	(rover for Mars 2003 and 2005 missions)
BSL	biosafety level
CAPTEM	Curation and Analysis Planning Team for Extraterrestrial Materials
CDC	Center for Disease Control (Atlanta, GA)
cpm	counts per minute
DIII	Delta III
duricrust	fine-grained debris close to the surface cemented to form a crust a few millimeters thick; probably a cement of soluable mobile salts similar information to the caliche crusts on dry lake beds
EEV	Earth Entry Vehicle
ERV	Earth Return Vehicle
HEPA	high efficiency particulate air (filter)
ICBC	Interagency Committee on Back Contamination
ISS	International Space Station
JSC	Johnson Space Center (Houston, TX)
LDEF	Long Duration Exposure Facility
LEO	low Earth orbit
LPI	Lunar and Planetary Institute (Houston TX)
LPS	Lipopolysaccharides
LR	labeled release
LSAPT	Lunar Sample Analysis and Planning Team
MAV	Mars Ascent Vehicle
metazoa	many celled animals as distinct from single-celled protozoa
MSHARP	Mars Sample Handling and Requirements Panel
NIH	National Institutes of Health
NRA	NASA Research Announcement
NRC	National Research Council

ppb	parts per billion
PET	Preliminary Examination Team
protozoa	single-celled or acellular animals
SCF	sample curation facility
SEM	scanning electron microscope
SETI	Search for Extraterrestrial Intelligence
SRF	sample receiving facility
SRM	sample return mission
TBD	to be determined
TGISR	Task Group on Issues in Sample Return (National Research Council); also referred to as the NRC Task Group
Tetrahymena	Tetrahymena thermophila is a unicellular, ciliated freshwater protozoan. It is well suited for research in that cells can be grown overnight to densities of 100,000 cells per ml or more. These cells exhibit a wide repertoire of behaviors that can serve as model systems for investigative analysis.
ULPA	Ultra low penetration air (filter)
UV	ultraviolet
XRFS	X-ray fluorescent spectrometry

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	04/19/99	NASA Technical Memorandum

4. TITLE AND SUBTITLE
Mars Sample Handling and Requirements Panel (MSHARP)
Final Report

5. FUNDING NUMBERS
C-NAS7-1407
050000-02.01.001

6. AUTHOR(S)
M.H. Carr, D.J. McCleese, J.L. Bada, D.D. Bogard, B.C. Clark, D.DeVincenzi, M.J. Drake, K.H. Nealson, J.J. Papike, M.S. Race, D. Stahl

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Jet Propulsion Laboratory
California Institute of Technology
4800 Oak Grove Drive
Pasadena, CA 91109-8099

8. PERFORMING ORGANIZATION REPORT NUMBER
NASA/TM-1999-209145

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
National Aeronautics and Space Administration
Washington, DC 20546-0001

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
NASA/TM-1999-209145

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
In anticipation of the return of samples from Mars toward the end of the first decade of the next century, 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 nonhazardous; and (3) sample receiving, curation, and distribution. This report summarizes the findings of that panel. 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.

14. SUBJECT TERMS
space biology, planetary protection, sample preservation, tracers, Mars, sample return, Mars sample return

15. NUMBER OF PAGES
86

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited