MARS SAMPLE QUARANTINE PROTOCOL WORKSHOP

Proceedings of a Workshop
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PREFACE

In 1996, several NASA-sponsored studies were underway to look at various aspects of a Mars Sample Return (MSR) mission. One of these studies, performed by the Mars Exploration Long Term Science Working Group (MELTSWG), looked at many issues for MSR including Planetary Protection (PP), both forward and back contamination aspects. One outcome of the study was the realization that little detailed information existed in certain PP-related areas that could be used by mission planners to more accurately design and cost MSR mission concepts. Therefore, the MELTSWG group recommended that NASA fund an effort to look at these PP issues in more detail.

A joint Ames Research Center-Jet Propulsion Laboratory-Johnson Space Center proposal was prepared, submitted to NASA Headquarters, and funded. It contained 5 tasks, each of which dealt with a specific PP element for a MSR mission: 1) definition of the environmental impact review process; 2) determination of outbound PP requirements; 3) examination of sample containment technology; 4) development of concepts for ensuring that uncontained Mars material would not be brought to Earth; and 5) development of guidelines for returned sample containment and quarantine analysis. The Workshop on Mars Sample Quarantine Protocol was conducted to address the fifth objective; it was convened at NASA Ames Research Center, June 4-6, 1997.

INTRODUCTION

The Mars Sample Quarantine Protocol (QP) Workshop was convened to deal with three specific aspects of the initial handling of a returned Mars sample: 1) biocontainment, to prevent uncontrolled release of sample material into the terrestrial environment; 2) life detection, to examine the sample for evidence of live organisms; and 3) biohazard testing, to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere (see Workshop Agenda in Appendix B). Experts in each of these areas from a variety of institutions were invited to participate (see Participants List, in Appendix C).

In order to constrain the scope of the Workshop, several assumptions were given. These included: 1) the Mars Sample Return (MSR) mission will be launched in the 2005 opportunity; 2) the mission will return samples from biologically interesting sites based upon data to be returned from the Mars Surveyor Program missions in 1996, 1998, 2001, and 2003; 3) in a nominal mission, the sample will not be sterilized prior to return to Earth; 4) the amount of sample available for quarantine tests will be a small fraction of the total amount returned; and 5) biocontainment of the unsterilized sample will be maintained until quarantine testing for biohazards is accomplished.

During the first part of the Workshop, several tutorials were presented on topics related to the workshop in order to give all participants a common basis in the technical areas necessary to achieve the objectives of the Workshop. For the second part of the Workshop, the participants were divided into three Subgroups to address each of the three sample handling issues: biocontainment, life detection, and biohazard testing. The Subgroups discussed the major issues in each area, developed recommendations and guidelines as appropriate, and identified research and technology development needs.

This document is the final report for the QP Workshop. It is organized along the lines of the Workshop itself, with the first part comprised of summaries of the background tutorials and the second part comprised of the reports from the three Subgroups. It is hoped that this report will 1) assist NASA's Planetary Protection Officer in identifying high-priority research and technology efforts that need to be undertaken to prepare for Mars sample return, 2) provide guidance to MSR mission planners and designers, and 3) serve as input to advisory groups and other entities who will ultimately establish sample return handling policy, requirements, and implementation.

BACKGROUND TUTORIALS

The background tutorials were designed to address several key issues that would be important to develop recommendations for returned sample handling. These included understanding the chemical and physical properties of the returned Mars samples, defining a representative mission design for Mars sample return, reviewing existing guidelines to prevent back contamination, and reviewing the lunar sample quarantine testing protocol. In addition, tutorials were presented on modern techniques for containment of pathogenic organisms, for identification of unknown biological entities, and for determining pathogenicity of sample constituents. This section contains summaries of each tutorial prepared by the presenters.

Mars Environment

Benton Clark
(Lockheed/Martin Aerospace)

The current nominal martian environment, relevant to the possible existence of microorganisms, is significantly different from virtually any specific environment or microenvironment on Earth. However "oases," unique isolated or microenvironments, may exist which could mimic environments that organisms of known metabolism may be able to exploit.

Mars is smaller than Earth, only one-tenth the mass, but with a surface area approximately the same as that of the land area on Earth. The bulk density of Mars is only 3.9 g/cm^3 compared to Earth's 5.5 g/cm^3. This indicates a smaller, probably less dense core and a general bulk composition enriched in lighter elements. The abundances of light elements at the surface is actually lower, at least for H and O (in H_2O), for N (in N_2), and most likely for C.

Surface gravity on Mars is 3/8 of Earth's. Mars' diurnal period of 24 hours 37.4 minutes (defined as a "sol") is strikingly close to Earth's current diurnal period, although there is no known fundamental reason for this coincidence. Mars' obliquity (axial tilt relative to the plane of motion around the Sun) is 25 °C, which is close to Earth's value. Thus, seasonal effects due to geometrical variations in insolation as a function of latitude, are similar. Mars' obliquity is quasi-chaotic, varying from values that may approach 0 °C to excursions much above present values, over time scales of 10^5 to 10^6 years. These changes can cause large and not easily predicted changes in atmospheric pressure, circulation, and climate.

Because of a significant orbital eccentricity, the southern latitudes have both hotter summers and colder winters than do the northern latitudes. Mars ranges from 38% to 67% farther from the Sun than the Earth, which results in one-third to one-half the solar insolation. As a consequence of the astrodynamics of this further distance, the length of the martian year is 687 days, with seasons approximately twice as long as seasons on Earth.

Mars has two satellites, Phobos and Deimos. They are tiny compared to Earth's moon and have no consequent gravitational effects. Their spectral properties resemble those of D asteroids, which

are thought to be organic rich, and they may in fact have provided material which entered the martian atmosphere and contributed to the composition of martian soil. There is little or no organic material in martian soil, a key result found by the Viking lander mission experiments that included pyrolysis gas chromatograph/mass spectrometer (GC-MS) instruments specifically designed for high sensitivity (1 ppm or less) analyses capable of detecting a wide variety of organic compounds. The virtual lack of organics detected by these sophisticated GC-MS instruments has been interpreted as due to the oxidation of all organics on Mars, whether endogenous or exogenously delivered. The highly oxidizing compounds present (not only O_2, but also O_3, $O\bullet$ radical, and the extremely strongly oxidizing hydroxyl radical) are the result of UV-induced photochemistry.

Mars has no exposed bodies of liquid water, although it has been hypothesized (based on very circumstantial evidence) that there may have been lakes and even an "ocean" on Mars in its early history.

The atmosphere of Mars has a nominal pressure of 6 millibars, much less than Earth's one bar. Pressure change as a function of altitude has a 30% annual variation due to condensation of its most predominant compositional component, CO_2, onto the polar caps during local wintertimes. Even larger variations in CO_2 pressure must be expected during obliquity excursions, with the atmosphere being drawn down to very low levels and possibly increased at other times.

The atmospheric composition includes 95% CO_2, N_2 (2.7%), argon (1.6%), O_2 (0.13%), and much smaller amounts of other constituents (CO at 70 ppm, H_2 at 30 ppm, and OH radical at 1 ppt). In addition, relative humidity at the nighttime lows can reach saturation, although the total H_2O partial pressure is typically 1 microbar (160 ppm) or less. This is a much drier, lower dew point, than the driest of the Antarctic Dry Valleys.

The geomagnetic field of Mars is tiny or nonexistent. As a result, galactic cosmic rays and the particles from solar flares are not magnetically deflected. Even though relatively thin, the martian atmosphere's total path density at zenith is nominally 16 g/cm^2, with much thicker shielding against ionizing radiation at the larger angles (which accounts for most of the solid angle of exposure). As a result, radiation doses at the surface of Mars are not severe, and in fact much lower than on the surface of the moon. Cosmic ray doses are less than 6 rad/yr even at 4-km altitude. Solar flare doses are even less since the worst case solar flare recorded since the beginning of the space program would have produced less than 20 rad at the surface of Mars. The atmosphere does not, however, shield the solar ultraviolet rays, with UV down to 200 nm penetrating effectively to the surface.

The surface of Mars, as studied by Viking and Pathfinder, is made up of soil-like dust deposits, drifts (dune-like accumulations of fine material), duricrust (hardened soils), peds (fragments of duricrust), and rocks. The particulates in the soil may be extremely fine grained – approaching the micron to few micron size of the suspended dust, which is much finer than lunar and most terrestrial soil particulates, except for loess and clays.

The early Mars environment is an enigma. With widespread evidence of extensive water erosion, the case for an early "wet" environment on Mars is compelling, although far from proven. The

major controversies on interpretations revolve around "how warm, how cold, and how wet." For example, geomorphological evidence of aqueous events and episodes on Mars may be explicable by ground water processes near freezing, and by catastrophic, episodic floods. Other interpretations appeal to precipitation, i.e., rain and runoff; formation of rivers, perhaps ice-encrusted; and other more terrestrially common phenomena. Average high temperature regimes are also uncertain owing to the faint early Sun and the calculated instability of greenhouse gases under photochemical destruction by UV for atmospheres with only minor ozone shielding.

Restricted, confined, and unique environments on Mars are a distinct possibility. Current surface average temperatures are cold (–55 °C for deep equatorial average temperatures), surface solar heating only exceeds 0 °C for the topmost millimeters of surface soils. There may be geographically restricted sources of significant heat (magmatic intrusions are one possibility). Another is meteoroid impact, which results in buried heat that can persist for 10^4 to 10^5 years. If the soils are clays or other hydrous minerals, there may be significant trapped H_2O available for hydrothermal environments.

The geothermal gradient for a planet provides a temperature environment that is above the triple point of water of 0 °C at some depth. For Mars, this depth can only be estimated and is expected to occur at a depth of a few kilometers. The recent discoveries of the extensive deep microbiological niches in the Earth lends credence to a similar hypothesis for Mars. Deep underground biotic activity could be shielded from the highly oxidizing compounds in the martian atmosphere, but cannot depend on the Sun as an energy source and must be accommodated by a geochemical energy source which is sufficiently abundant to sustain a biota over geologic time scales.

Chemical and Biological Studies of Mars Meteorites

Carlton C. Allen
(Lockheed/Martin Engineering and Science Services)

Twelve meteorites, with a total mass of 80 kg, are accepted as having come to Earth from the planet Mars. The meteorites are tied to a common parent body by the ratios of oxygen isotopes in their silicate minerals. These ratios are concordant as well as distinct from those of terrestrial and lunar rocks, and other meteorites. All twelve of the meteorites are igneous rocks, and eleven are geologically young, which indicates that they originated on a planet-sized body. Several of the meteorites contain trapped gas that closely match the composition of the martian atmosphere. Models of hypervelocity impacts on planetary surfaces indicate that a small percentage of the near-surface target material can be ejected into space. These data combine to provide extremely strong support for a martian origin of the meteorite suite.

Chemical Studies

The Mars meteorites have been studied with the full range of modern analytical techniques, and a wide variety of data has resulted. These data comprise the 'ground truth' for remote sensing

studies of Mars, and the 'reality check' for chemical and mathematical modeling. The analytical tests, and the type of information gained from each, are summarized as follows:

- Petrography (optical and electron microanalysis)
    - Solidification conditions
    - Alteration conditions
    - Shock effects
    - Atmospheric entry effects
- Chemical composition (neutron activation analysis, electron and proton microanalysis, mass spectrometry)
    - Whole rock composition
    - Individual mineral components
    - Variations within minerals
    - Alteration mineralogy
    - Trapped gas and water
    - Temperatures and pressures of crystallization and alteration
- Isotopic dating
    - Crystallization age
    - Shock age
    - Space exposure age
    - Terrestrial exposure age
- Stable isotope fractionation
    - Parent body identification
    - Temperature and chemistry of alteration
    - Atmospheric history of Mars
- Paleomagnetism
    - Constraints on martian core
    - Temperature constraints on alteration
- Melting experiments
    - Temperature, pressure, and redox conditions during crystallization

Biological Studies

The 1996 publication of possible evidence for relic biogenic activity on Mars was based on detailed studies of the meteorite ALH 84001. Since that time, this Mars meteorite and several others have been subjected to a greater variety of tests relevant to possible evidence for life in these samples. A joint NASA/NSF program to support intensive biological studies of Mars meteorites commenced during 1997. These biological studies, and some of their results, can be summarized as follows:

- Laser desorption/ionization mass spectrometry
- Detection and location of organic compounds
- Microscopy (optical, electron, atomic force)
    - Possible biominerals
    - Possible microfossils
    - Possible biofilms

- Culture media
- Isotope fractionation
    Possible biological signatures (C, O)
    No biological signature (S)
- Amino acid analyses

<u>Significance of Mars Meteorites for Sample Return</u>

These twelve meteorites are representative of a much larger body of martian material which has already come to Earth. The Mars meteorites contain alteration minerals that would have been destroyed by high temperatures, indicating that the ejection process did not thoroughly heat every meteorite. While in space the meteorites were cold-soaked. The radiation environment of space does not destroy organic compounds in rocks (witness amino acids in carbonaceous chondrite meteorites). The Mars meteorites were not sterilized by entry into the Earth's atmosphere; only the outer 5–10 mm was heated significantly. If any of these rocks harbored life on Mars, that life could have survived transfer of the rocks to Earth. These unsterilized, random samples of Mars have been on Earth for at least 100,000 years and in contact with people for nearly 200 years. However, none of this obviates the statutory requirements for containment and testing of returned martian samples.

Mars Sample Return Mission Design[1]

Mark Adler
(Jet Propulsion Laboratory)

The impetus for this Workshop comes from NASA and the Administration's request of funds from Congress for an ambitious, long-term Mars exploration program that includes the return of samples from Mars. This paper will describe that program, the science strategy behind it, and key aspects relevant to this Workshop.

Late in 1996, two spacecraft were launched to Mars: Mars Pathfinder, which landed on July 4, 1997 and deployed the first Mars rover, Sojourner, and Mars Global Surveyor, which entered into orbit about Mars in September 1997 and has begun to characterize Mars with an array of instrumentation. These are the first in a continuing series of missions, called the Mars Surveyor Program, that NASA will send to Mars, two missions every Earth-Mars launch opportunity – approximately every 26 months. Another orbiter and lander were built and launched in the 1998/1999 opportunity. This orbiter will continue the global survey with different instrumentation, and its lander will explore the near-polar regions with an arm to deliver soil samples to its instrumentation.

In August 1996, a significant announcement brought the attention of the world to Mars. As described earlier by Carlton Allen, we already have the first sample returns from Mars provided to us by nature – the Mars meteorites. A team of researchers at Johnson Space Center and Stanford

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[1] Between the dates of this Workshop and submission of this report for publication, Mars exploration plans have been changing. Some of the final mission details may be different from those reported here.

University found in one of those meteorites what they believe to be evidence of ancient life on Mars. Their announcement precipitated a redesign of the Mars Surveyor Program with increased funding beginning with the 2001 opportunity.

The funding level of this new program is approximately $250 million per year, including launch vehicles and operations. In addition to that, significant funding is planned to provide the necessary technology development to enable the missions described below.

The science strategy behind the planning of the 2001 and beyond missions is guided by a search for evidence of life. The search is based on the assumption that of the key ingredients for life (liquid water, organic molecules, and a source of energy) at least two are evidenced on ancient Mars, liquid water, and a source of energy. The search for three distinct environments on Mars is planned based on the extrapolation to Mars of theories for the origin of life on Earth. These are ancient surface water deposition sites, which have been directly imaged during prior space missions, projected ancient groundwater environments, and projected modern ground water environments. The strategy is to look for evidence of life for all three environments in the hope that at least one will be fruitful.

Equally important to the search for life is the understanding of the environmental requirements for life, especially with respect to water. The history and evolution of water on Mars – where the water was, where it went, and where it is today, will be the foundation of this understanding. The 1996 and 1998 missions will begin to lay this foundation.

So how does one search for what may be rare evidence of life on a planet whose surface area is about the same as the land mass area of Earth? And how does one find and confirm this potentially controversial discovery even if it can be found? The most sensible implementation to address these questions is to bring to Earth laboratories very carefully selected samples from the surface of Mars. Not only is it extremely cost effective to do analyses using the fully equipped Earth laboratories, as opposed to occasionally sending limited instrumentation to Mars, but it may be essential in order to answer challenges to claimed scientific conclusions by performing new investigations on the samples. Such challenges would be expected and even necessary for extraordinary claims such as evidence of extraterrestrial life. This pattern of research is proceeding on ALH 84001.

The missions to embark on the search for past or current biologic activity consists of three elements, repeated over time to investigate the different environments. Those elements are: 1) site selection from global mapping and targeted mapping orbiters, 2) sample selection with rovers or digging landers, and 3) sample returns to bring selected samples safely to Earth for investigation.

Site selection for sample returns began effectively with the Viking orbiters of the late 1970's, and will continue with the Mars Global Surveyor orbiter and the 1998 orbiter. This data will provide high-resolution imagery of the planet, altimetry, thermal emission spectra, and atmospheric water information. An orbiter to be launched in early 2001 will complete the first set of Mars global data with a gamma ray and neutron spectrometer to look for evidence of water molecules in the upper

surface, and will carry a mineralogy and morphology investigation specifically to provide the information needed to find good candidate sites for the hypothesized environments for life. The first round of sample selection missions will be long-range, long-life rovers launched in 2001 and 2003 to two different sites. The 2001 rover's site will be selected with the benefit of the orbital data up to and including the 1998 orbiter. The 2003 rover's site selection will have in addition the benefit of the data from the 2001 orbiter. Each of these rovers will have a lifetime of at least one Earth year and a range of several kilometers. They will have the instrumentation and mechanisms to examine sections of rock by appearance and spectra, and to store selected samples in a cache for later retrieval.

In late 2004 or mid-2005, the first Mars sample return mission will be launched to one of the two sample selection sites to meet up with either the 2001 or 2003 rover. Which rover will depend on which site is deemed to have the most scientifically interesting sample. The sample return lander will touch down a short distance away and dispatch its own rover to fetch the sample cache from the possibly non-operational rover that collected the sample. The sample cache will be put in a sample container. That container may have some martian regolith added if that was not already part of the cached sample, and the container is then welded shut to contain martian atmosphere which is also an important component of the returned sample. That sample container is then returned to the sample return lander where it is placed into a rocket that is launched into orbit around Mars. Waiting in orbit is the Earth return vehicle, also part of the sample return mission, that will rendezvous with the sample rocket in orbit and transfer just the sample container to the Earth re-entry vehicle, which is carried on the Earth return vehicle. The Earth return vehicle exits Mars orbit for Earth, and on approach targets the Earth entry vehicle to the selected landing site on Earth. Upon releasing the entry vehicle, the return vehicle changes course to avoid the Earth. The sample lands in mid-2008.

This sequence of site selection, sample selection, and sample return is envisioned to continue for two more sample return missions in order to investigate the martian environments which have the best chance of finding evidence of life. The last of the three samples is returned in 2016, twenty years after the initiation of the program in 1996.

The mission sequence consisting of a sample selection rover, a sample return mission, and the subsequent analyses of samples is considered a life detection experiment. As such, the effectors, instruments, and container on the rover, essentially anything that comes in contact with the samples, must be void of Earth biology to avoid the detection of Earth life in the returned samples. The same is true for those elements of the sample return mission that could contaminate the sample.

Most relevant for this Workshop is the additional requirement to avoid any possibility of inadvertently releasing martian material upon entering Earth's atmosphere and landing on Earth. This is required to avoid the vanishingly small, but non-zero risk of viable martian biology that might threaten the Earth's ecosphere. The sample will remain in containment until it is determined that it poses no hazard. Some science investigations can be performed on the sample while it is in containment. However, it is extremely desirable to distribute the sample as soon as possible after return to the broadest scientific community. Only through this release can the best instrumentation and widest scientific discourse be applied to answer the truly difficult questions

that these samples are expected to represent. The assurance of containment on Earth and the criteria for release are as critical to the success of our Mars exploration program as the return of the sample itself.

To meet the requirement of no inadvertent release of uncontained martian material on Earth, an externally clean sample container with Mars material securely sealed inside must be transferred in Mars orbit from the dirty sample return ascent rocket to the clean orbiter. Then the sample return ascent rocket is discarded leaving only clean elements and contained martian material to be returned to Earth. The containment is monitored after the transfer and during the trip back, perhaps by monitoring the pressure and temperature of the martian gases inside, to verify containment before committing to the return of the sample to Earth. If the sample is not adequately contained, it is not returned. The Earth entry vehicle and sample container are designed to assure that sample containment will not be breached on entry or impact.

The systems for this clean transfer from dirty components have not yet been designed. However, the concept is for the sample container and its lid to be double-walled, with the outer part discarded during the transfer in orbit, leaving the inside part clean. The inevitable seam between the two could have a pyrotechnic charge to mechanically remove the small amount of dust at that boundary. A large shield would protect the return vehicle from the dirty ascent rocket during the transfer, and that shield would be discarded along with the rocket after the transfer is completed.

An alternative strategy of heat "sterilizing" the sample before return is not envisioned. The difficulty of defining and generating sufficient heat for an adequate time to sterilize a biology whose nature is inherently unknown is a problematic task. The potential exists to destroy critical information of the sample.

It is often asked whether the sample should be brought to the Space Station, or some orbital facility, instead of landing it directly on Earth, thereby allowing analysis of the potential exposure consequences before bringing it to Earth. While that sounds reasonable at the outset, one quickly discovers that there are several significant difficulties. First, in order to put the sample into orbit, it would have to be "aerocaptured," which means using something much like the direct entry vehicle to plow into the atmosphere, whereby the atmosphere slows it down enough to enter into a low Earth orbit. This is a very sophisticated technological operation, requiring accurate navigation and hypersonic maneuvering in the atmosphere. Even a small error can result in the vehicle landing on Earth in an uncontrolled manner and to an unpredictable landing site.

Second, assuming it can reach orbit and be retrieved by the Space Station, the sample and the Space Station are now in a low Earth orbit. A failure in the Space Station could result in an evacuation and the possibility of a Skylab-like reentry soon after.

Third, the facilities needed on the Space Station to perform the necessary analyses in containment would be extensive, and still would be inferior to the equipment and containment available on Earth owing to the limited mass available. Putting aside the expense of such facilities, if there were a failure of containment, then one would have some rather difficult questions to answer about how to deal with the exposed personnel.

Finally, as will be discussed at this Workshop, Earth laboratories routinely handle biological entities known to be dangerous. These processes and procedures properly applied to samples from Mars would provide a level of safety already deemed adequate for the protection of people and the ecosystem from biological hazards. In summary, Earth is a much safer place to bring the samples than any orbiting facility.

A twenty year exploration of Mars has been planned whose strategy and funding level lead to the first return of fresh samples arriving at Earth in 2008, with at least two subsequent returns. These samples are part of a life detection investigation, and as such cannot be considered to be risk-free to Earth. They must be contained and carefully investigated before release to the general scientific community. Significant precautions will be taken to assure that the samples are not released in the process of delivery to Earth. To land a contained sample on Earth is considered to be far safer and efficient than the difficult operations needed to put the sample into Earth orbit and have it contained and analyzed in an orbital facility. Both the containment of the samples on Earth and the careful and deliberate release of the samples to the scientific community are critical to the success of our Mars exploration strategy.

SSB Guidelines for Prevention of Back Contamination from Mars Sample Return

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Context

In late 1995, NASA requested that the National Research Council Space Studies Board (SSB) examine and provide advice on planetary protection issues related to possible sample return from near-Earth solar system bodies. The 1997 report of the SSB's Task Group on Issues in Sample Return [Space Studies Board 1997] addressed questions of scientific, technological and policy concerns and made recommendations on how to ensure that any sample returned to the Earth from elsewhere in the solar system would have no adverse effects on the Earth's biosphere. The findings and recommendations of the SSB Task Group provide important background information for this Workshop as it deals with containment, life detection, and biohazard testing issues for Mars sample return missions.

Summary of Findings

The charge to the SSB Task Group on Issues in Sample Return was broad and ambitious, covering five specific areas of concern:

- The potential for a living entity to be included in a sample to be returned from another solar system body, in particular Mars;
- The scientific investigations that should be conducted to reduce uncertainty in the previous concern;
- The potential for large-scale effects on the environment resulting from the release of any returned entity;

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- The status of technological measures that could be taken on a mission to prevent the unintended release of a returned sample to Earth's biosphere; and
- Criteria for controlled distribution of sample material taking note of the anticipated regulatory framework.

In developing its report, the Task Group deliberately focused on providing high level policy recommendations – deferring details of implementation to NASA and appropriate experts at a later time. The report is prefaced with the following key findings that were formulated after lengthy discussions by the Task Group:

- 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;
- While contamination of the Earth by putative martian microorganisms is unlikely to pose a risk of significant ecological impact or other significant harmful effects, the risk is not zero;
- 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.

Despite the low risks anticipated from returning samples from Mars, the Task Group felt that a cautious approach should be taken by including appropriate planetary protection measures for a number of important reasons: 1) to protect the Earth from inadvertent cross contamination; 2) to preserve the integrity of returned materials for scientific interpretation; 3) to meet legal and regulatory obligations; and 4) to reassure the public that adequate environmental, health, and safety measures have been taken.

The conservative approach to planetary protection suggested by the Task Group recommends that each returned sample should be assumed to contain viable exogenous biological entities until proven otherwise. Moreover, specific additional recommendations were made in the following three major areas related to eventual implementation:

1. Sample Return and Control
   - Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.
   - 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.
   - Integrity of containment should be maintained through reentry of the spacecraft and transfer of the sample to an appropriate receiving facility.
   - 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.

- 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.

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

3. Program Oversight
   - A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample return missions. The panel should be in place at least one year prior to the establishment of the sample receiving facility (at least three years prior to launch).
   - An administrative structure should be established within NASA to verify and certify adherence to planetary protection requirements at each critical stage of a sample return mission, including launch, reentry, and sample distribution.
   - Throughout any sample return program, the public should be openly informed of plans, activities, results, and associated issues.

Finally, the Task Group emphasized the value of research to reduce uncertainty and to aid in eventual implementation. Specifically identified research of potential merit included NASA's current stepwise Exobiological Strategy for Mars Exploration [NASA 1995], and further study of terrestrial extremophiles and martian meteorites. Research and development was also urged in key areas in order to advance the technologies and methodologies for sample containment, cross contamination avoidance, sample sterilization, in-flight verification of containment, in-flight sterilization, and sample handling and preservation.

Returning martian samples to Earth will be a momentous scientific and technological advance that must be done carefully and prudently. Even though the risks are low, the stakes are very high. Planetary protection measures are justified by both what we know and what we don't know about Mars and its potential for harboring life. Early integration of planetary protection measures in mission planning is recommended as a way of increasing effectiveness, limiting costs, and satisfying legal and regulatory requirements.

References

Exobiology Program Office, National Aeronautics and Space Administration, *An Exobiological Strategy for Mars Exploration*, NASA SP-530, Washington, D.C. (1995).

Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C. (1997).

Lessons Learned During Apollo Lunar Sample Quarantine and Sample Curation

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A total of 12 men set foot on the Moon at 6 sites and returned to Earth 382 kg of lunar rocks and soil, comprised of 2196 individual specimens. The Apollo story of sample containment and preservation serves to illustrate the viewpoints of the various groups involved, the time required for integration and implementation, and the balancing of technical requirements for containment versus sample preservation.

Three groups, charged with different responsibilities, interacted during the lunar material quarantine and sample curation. NASA mission managers and engineers were focused on meeting the schedule and executing the missions. The Interagency Committee on Back Contamination (ICBC), an advisory committee to NASA, was charged with prevention of back contamination and endeavored to make sure that no destructive organisms were introduced into the Earth's biosphere by returned lunar material. Another advisory committee, the Lunar Sample Analysis and Planning Team (LSAPT) was concerned with the care and distribution of lunar rocks and soils and worked to prevent terrestrial contamination of the samples and to preserve the samples' scientific value. The very short time constraint on Apollo was a principal cause of problems encountered in executing strict containment, particularly with astronauts and spacecraft.

The ICBC, comprised of members from the U.S. Public Health Service, Departments of Agriculture and Interior, the National Academy of Sciences, and NASA, was officially established only 41 months before the ultimate launch of Apollo 11! The ICBC had the final authority over release of lunar samples and astronauts.

The crews and samples from Apollo 11, 12, and 14 were subjected to quarantine protocols, which attempted to expose lunar material to at least one representative species from each phylum of terrestrial plants and animals. Each class of protocol had a decision tree for quarantine testing or sample release recommendations, but all were similar: if any differences occurred between exposed group and control, that were not explicable as terrestrial contamination, then second order testing was recommended; otherwise release of samples was recommended. No evidence of replicating agents was found in the test systems used, and all samples were released unconditionally. Quarantine ended with Apollo 14, after which distribution of Apollo lunar samples

was left solely under the purview of the scientific advisory committee LSAPT whose concern was scientific preservation of samples.

The state-of-the-art Lunar Receiving Laboratory (LRL) at the Johnson Space Center comprised 8,000 m^2 of sample receiving laboratory, biological quarantine testing facilities, crew isolation area, gas analysis laboratory, and radiation-counting laboratory. Processing of lunar samples in a high vacuum environment was deemed a requirement by the planetary science community because it preserved lunar-like conditions, as much as possible, and because no one knew how lunar materials would react with various gases. Cost of construction, equipping, and operating the LRL in 1969 and 1970 was about $24 million [Piland 1969] (which translates to ~$125 million in 1997-98 dollars). At the height of quarantined missions 200 technicians worked in 3 shifts per day supporting 100 NASA civil servants and visiting scientists.

At the conclusion of the Apollo 17 sample preliminary examination in 1973, all of the samples were moved to a facility more suited for sample curation and preservation. Differences in containment versus sample purity technical requirements made the LRL less suitable. A building especially designed to keep samples pure was completed in 1979 and operated by less than 20 people, at substantially lower cost than the LRL.

Use of lunar sample for quarantine testing competed with its use for science investigations and prudent curation for future studies. One guiding principle was minimization of sample consumed in quarantine testing. Where possible, lunar material destined for quarantine testing was taken from the residue of fines in the bottom of sample containers, since this material was of less scientific interest. Out of a total 98.189 kg from Apollo 11, 12, and 14, 2% was allocated for quarantine testing proportioned as follows: residue fines 59.0%, fines 31.3%, cores 6.1%, and rocks 3.6%.

In 1967 the Lunar Receiving Laboratory had 4 stated functions: 1) distribution of samples to the scientific community, 2) perform time-critical sample measurements, 3) permanent storage under vacuum of a portion of each sample, and 4) quarantine testing of samples, spacecraft, and astronauts [McLane et al. 1967]. In contrast, today the purpose of curation of extraterrestrial materials at Johnson Space Center is to: 1) keep the samples pure, 2) preserve accurate historical information about the samples, 3) examine and classify samples, 4) publish information about newly-available samples, and 5) prepare and distribute samples for research and education.

Lessons were learned in setting quarantine policy and sample handling planning, as well as in the technical approach to sample handling, depending on the area of concern. Such lessons included: 1) initiate planetary protection and sample preservation planning early in mission design; 2) place responsibility for back contamination and sample preservation at high management levels; 3) allow time for proper implementation of back contamination and sample preservation requirements; 4) reduce the magnitude, and thus cost, of quarantine and curation by careful pacing and careful planning of what to do in quarantine mode and what not to do in quarantine mode; and 5) build a scientific foundation for mutual respect for quarantine and sample preservation.

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Lessons in technical approach: 1) devise a plan to minimize conflicts in protocols for quarantine versus sample examination and preservation; 2) strive to minimize the amount of sample required for biohazard evaluation through improved technology; 3) strive to minimize the cost of quarantine through improved technology and timing of non-quarantine functions; and 4) reserve a portion of the sample for future studies.

References

McLane, J.C., Jr., E.A. King, D.A. Flory, K.A. Richardson, J. P. Dawson, W.W. Kemmerer, and B.C. Wooley, "Lunar Receiving Laboratory," Science 155:525-529 (1967).

Piland, J., Lunar Receiving Laboratory Cost Recap; Data Furnished to John Madden, NASA Headquarters for Congressional Inquiry, Manned Spacecraft Center, Houston, TX, USA (1969).

Modern Techniques for Containment of Pathogens

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The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) facility at Fort Detrick in Frederick, Maryland has unique capabilities to support its mission to develop vaccines, antiviral drugs, and diagnostic reagents for etiologic agents requiring containment. Coupled with our biological and chemical safety philosophy, USAMRIID is ready to support other missions requiring maximum containment for the analysis of samples.

USAMRIID's mission is to conduct research to develop strategies, products, information, procedures, and training for medical defense against biological warfare agents and naturally occurring infectious diseases of military importance that require special containment. The unique talents and capabilities of USAMRIID include a scientific staff of about 100 postgraduate level professionals with expertise in the biological, chemical, medical, and laboratory animal disciplines. USAMRIID's facilities include 23 laboratory suites containing over 10,000 ft^2 of Biosafety Level 4 (BSL-4) and 50,000 ft^2 of BSL-3 space, a sixteen-bed clinical research ward, a four-bed BSL-4 patient care containment suite, a BSL-4 containment clinical laboratory, and specially designed engineering systems.

Most known infectious agents have been classified by the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) by their hazard levels or Biosafety Levels (BSLs). Once assessed, appropriate containment levels and techniques can then be selected. There are four major levels of hazard containment:

1. Biosafety Level 1 (BSL-1) practices, requirements, and safety equipment are appropriate for facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. E. coli is an example of an agent of very

low hazard. BSL-1 facilities include undergraduate and secondary educational training and teaching laboratories.

2. BSL-2 practices, requirements, and equipment are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community. These agents are associated with human disease of varying severity.

3. BSL-3 practices, requirements, and equipment are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous moderate-risk or exotic agents where the potential for infection with aerosols is real and the disease may have serious or lethal consequences.

4. BSL-4 practices, requirements, and equipment are applicable to work with dangerous and exotic agents that pose a high individual risk of life-threatening disease. BSL-4 containment is required for high hazard agents characterized as highly virulent, of high mortality, and for which no vaccine or therapy exists. Examples are Lassa, Ebola, and Marburg viruses.

The BSL-1 through BSL-4 laboratories in USAMRIID meet or exceed the laboratory Biosafety Level criteria recommended in the CDC-NIH guidelines. For instance, the exhaust air from all BSL-3 laboratories is filtered through a high efficiency particulate air (HEPA) filter prior to discharge, and there is a requirement to take a wet shower upon exit from these laboratories. Most biocontainment areas at USAMRIID are at the BSL-3 level (total of about 50,000 ft^2).

The purpose of BSL-4 laboratory operations is to provide an environment where live, replicating agents of human disease can be studied in cell cultures and animal models. Characteristics of laboratory operations within this high hazard area include enforcement of safety regulations, staffing with conscientious workers, maintaining inventory control of infectious materials, and maintaining a specialized occupational health program. All work in BSL-4 laboratories is conducted in Class III biological safety cabinets or in spacesuits. Work surfaces are appropriately decontaminated after use. Aspects of BSL-4 laboratory engineering include laboratory location such as a separate building or sealed room with independent supply and exhaust, and restricted access. Construction is with reinforced concrete, and only chemically resistant coatings and non-hardening sealants for service openings are used. The laboratory perimeter is sealed to permit gaseous decontamination of the entire area and all surfaces are non-permeable. Each BSL-4 laboratory has a ventilated airlock with interlocking pneumatic sealed doors, and an interlocking double door pass-through autoclave. Microbial sterilization is achieved using heat (autoclave), chemicals (formaldehyde, bleach, and quaternary ammonium compounds), gases (formaldehyde and ethylene oxide), and radiation (ultraviolet light and gamma rays). Exhaust air is double HEPA-filtered and ventilation is monitored by magnehelic gauges. The exhaust system is interlocked with the air supply system to prevent positive pressurization.

USAMRIID is the only facility in the country capable of totally isolating and treating persons who have been exposed to a highly hazardous agent. USAMRIID has a special team, the Aeromedical Isolation Team, that is equipped to respond to local, continental, or worldwide medical emergencies. Their training is sustained through periodic practice drills.

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The goals of the USAMRIID biosafety program are to prevent injury, infection, and death of employees and the public, to prevent environmental contamination, to conform to prudent biosafety principles, and to comply with Federal, State, and local regulations and guidelines. Our task in the Safety and Radiation Protection Office is to preserve existing conditions. Everyone is healthy and it is our job to keep everyone that way.

In summary, some obvious hazards have been described as well as how the risks of these hazards are minimized. Complete or absolute safety cannot be provided but reasonable safety is provided. Policy is established and advice, guidance, limited training, and protective equipment is provided. USAMRIID has an integrated program of immunization, health surveillance, and medical management of illness. This program, combined with safety engineering features and equipment, is designed to reduce the risks associated with the unique research conducted at USAMRIID.

Trace Detection/Identification of Biological Entities in Martian Rocks and Soil

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Background

Our focus for detection of fastidious bacteria or their non-viable cell envelope components has been on techniques other than traditional culturing methods. It should be emphasized that bacterial culture techniques are a powerful and simple approach for the detection of life which should certainly be utilized in studies of putative martian life. However, we have no way of knowing the nutritional requirements of such life. Even many well known causative agents of human disease that have been studied for many decades cannot be cultured on conventional microbiological media. Indeed, in many instances they do not grow on any currently available laboratory media. Thus, even if a sample will not grow it still may contain life. Alternatively, non-culture based chemical approaches can detect life in the absence of laboratory growth.

Our first assumption for the purposes of this discussion is that life, if present on Mars, will contain a diverse collection of organic chemicals containing C, H, N, O, P, and S. It is reasonable, as a first approximation, to assume that martian life would be based on amino acids, sugars, fatty acids, and nucleotides some of which are polymerized respectively into proteins, polysaccharides, lipids, and nucleic acids, as they are on Earth. While it is possible that these martian chemicals may be identical to terrestrial chemicals it is equally possible that these compounds may be entirely distinct from those present on Earth. Thus it is our contention that methods selected should be of general applicability and capable of profiling the whole class of each type compound (e.g. L- and D- amino acids and/or sugars).

The Viking Mars missions recognized the power of mass spectrometry and utilized an approach based on pyrolysis. When performing pyrolysis, the sample is heated to several hundred degrees centigrade in the absence of oxygen. This shatters not only all polymers and oligomers present in

the sample but also the monomeric constituents (e.g. carbohydrates). Extremely low molecular weight volatile constituents are generated whose chemical structures bear little relationship to those of the parent molecules due to destructive scission and dehydration reactions. Catherine Fenselau noted in the introduction to her 1994 book *Mass Spectrometry for the Characterization of Microorganisms* [Fenselau 1994] that the use of pyrolysis to convert intricate endogenous chemical markers to small gaseous molecules is philosophically flawed and has been historically unproductive." This conclusion was not well understood at the time of the Viking missions but subsequently more than 20 years of experience have taught us that it is time to consider more modern mass spectrometry based approaches.

In the late 1970s into the 1990s, a number of state-of-the-art techniques based on "derivatization and mass spectrometry" have been successfully developed for trace detection of chemical markers for bacteria in complex clinical and environmental matrices. In derivatization, the monomers (e.g. sugars and fatty acids) are released from polymers in a chemically intact form by hydrolysis in acid or alkali. The monomers are volatilized by inhibition of ionic interactions and hydrogen bonding by conversion into a non-polar state (e.g. by acetylation). Originally such samples were analyzed by a combination of gas chromatographic separation of these complex mixtures into their components followed by mass spectrometric detection/identification (GC-MS). Recently, the power of these techniques has been improved dramatically using tandem (two dimensional) mass spectrometry for improved detection and trace identification (GC-MS-MS). From the mass spectra of these derivatized molecules, even in complex environmental samples it is possible to determine the structure of the original native molecules.

These latter (GC-MS and GC-MS-MS) methods are routinely available in a few specialist laboratories. It must be stressed that trace detection of microbial components in complex environmental matrices using mass spectrometry remains a novel, high technology research area. There are many well qualified microbiologists who possess knowledge of chemical/molecular composition of microbes and mass spectrometrists equally-skilled in the use of these highly sophisticated instruments. Analytical microbiology (a discipline on the interface between analytical chemistry and microbiology) combines expertise in these two diverse areas into a single interdisciplinary research program [Fox *et al.* 1990].

As an example, muramic acid is an amino-sugar that is unique to the bacterial cell wall peptidoglycan (PG) and serves as a universal marker for the presence of PG in environmental and clinical samples. In trace analysis of muramic acid in complex biological matrices, contaminating compounds are commonplace masking detection and causing false positives. Therefore, observing a chromatographic peak at the correct retention time (using a non-selective detector) does not constitute definitive identification. One may merely be detecting a co-eluting contaminant. When present at relatively high levels it is possible to categorically identify muramic acid in a chromatographic peak by the "mass spectrum." The mass spectrum is a chemical fingerprint which is characteristic for muramic acid. Alternatively, the mass spectrometer can be used as a selective chromatographic detector; in this "monitoring mode" the detection limit is much lower but absolute identification (by mass spectrum) is not possible.

Categorical identification at trace levels has awaited the development of more advanced instrumentation. The mass spectrometer is widely used as a "selective chromatographic detector"

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to ignore extraneous chromatographic peaks. In tandem mass spectrometry, the instrument further decreases nonspecific peaks by screening out background peaks twice. In the "identification mode" one can also obtain absolute chemical confirmation by means of a daughter mass spectrum (chemical fingerprint). The high resolution separating power of GC coupled with the exquisite selectivity of MS-MS detection eliminates essentially all extraneous peaks in the chromatograms. At the retention time for muramic acid, daughter mass spectra of peaks from environmental dust samples are identical to standard muramic acid.

High molecular weight oligomers and polymers (e.g. nucleic acids, proteins, and phospholipids) are not amenable to GC-MS and GC-MS-MS analysis. At atmospheric pressures, these molecules tend to adsorb/decompose in the injection port or GC column of the instrument. Alternatively, the molecules can be introduced into the near vacuum of the mass spectrometer as charged particles using the techniques of electrospray ionization or matrix assisted laser desorption. Both techniques have the potential for trace detection of polar and/or high molecular weight chemical markers for bacteria in complex matrices with minimal sample preparation.

The polymerase chain reaction (PCR) is an established alternative to culturing for trace generation of molecular markers of pathogens in environmental specimens. The rate limiting step in conventional molecular biology approaches is the detection of these PCR products by gel electrophoresis (which generally takes several hours). The first technology for the rapid (<10 min) identification of pathogenic bacteria based upon the combination of PCR and mass spectrometry has been recently developed as a result of a collaboration between Pacific Northwest National Laboratory and the University of South Carolina. In comparing a variety of PCR products from bacterial strains of known sequence with related products from strains of unknown sequence we have demonstrated that single base substitutions, deletions, or additions in PCR products can be readily recognized by the change in molecular weight as determined by MS analysis. Analysis of martian samples for specific genetic markers would not be recommended at this time. However, this approach demonstrates the ability to detect intact high molecular weight oligomers and polymers derived from bacteria with minimal sample work-up and no derivatization.

Transport of rock samples from Mars to Earth will happen in the not too distant future. The procedures for containment of such samples are under development. Methods for hazard determination (involving culture and toxicity testing) in cells and animals are well established for terrestrial microbes. Life detection by non-culture based techniques are only available in a hand-full of laboratories. Few of these laboratories currently focus on the study of extraterrestrial life and most will not readily respond to traditional requests for proposals from outside their disciplines (e.g. exobiology). Decisions need to be implemented in the near future on how to incorporate these technologies into the Mars mission planning. An extremely important logistical first step is whether there will be incorporation of appropriate mass spectrometry based instrumentation and expertise into the planned facilitates for containment and hazard evaluation. Alternatively, planning must be initiated now to determine how to release sterilized extracts to the wider scientific community. Such extracts must of course be clearly non-hazardous but also must retain the native structures found in chemical markers for life. Appropriate sterilization methods would most sensibly be developed by collaboration between exobiologists and those skilled in the area of analytical microbiology. Further, most environmental terrestrial samples which have been currently analyzed by modern mass spectrometry-based methods are teeming with life. Due to the

harsh conditions, martian samples may contain only small numbers of microbes. It needs to be proven that any methods selected are capable of quantitatively discriminating between terrestrial samples that clearly contain low- or high-levels of Earth organisms as negative and positive controls, respectively.

References

Fenselau, C., *Mass Spectrometry for the Characterization of Microorganisms* (1994).

Fox, A., L. Larsson, G. Odham, and S.L. Morgan, eds., *Analytical Microbiology Methods: Chromatography and Mass Spectrometry* (1990).

Bibliography

Fox, A., J.H. Schwab, and T. Cochran, "Muramic Acid Detection in Mammalian Tissues by Gas-Liquid Chromatography-Mass Spectrometry," *Infect. Immun.* **29**:526-531(1980).

Fox, A., S.L. Morgan, and J. Gilbart., "Preparation of Alditol Acetates and Their Analysis by Gas Chromatography and Mass Spectrometry," In: *Analysis of Carbohydrates by GLC and MS*, Bierman J., and G. McGinnis, eds., CRC Press, Boca Raton, FL p. 87-117 (1989).

Fox, A., K. Fox, B. Christensson, M. Krahmer, and D. Harrelson, "Absolute Identification of Muramic Acid at Trace Levels in Human Septic Fluids in Vivo and Absence in Aseptic Fluids," *Infect. Immun.* **64**:3911-3955 (1996).

Fox, A., M. Krahmer, and D. Harrelson, "Monitoring Muramic Acid in Air (after Alditol Acetate Derivatization) Using a Gas Chromatograph-Ion Trap Tandem Mass Spectrometer," *J. Microbiol. Meth.* **27**:129-138 (1996).

Muddiman, D.C., D.S. Wunschel, C. Liu, L. Pasa-Tolic, K.F. Fox, A. Fox, G.A. Anderson, and R.D. Smith, "Characterization of PCR Products from Bacilli Using Electrospray Ionization FTICR Mass Spectrometry," *Analyt. Chem.* **68**:3705-3712 (1996).

Shagholi, M., S. Ohorodnik, J. Callahan, and A. Fox, "Trace Detection of Underivatized Muramic Acid in Environmental Dust Samples by Microcolumn Liquid Chromatography-Electrospray Tandem Mass Spectrometry," *Analyt. Chem.* **69**:1956-1960 (1997).

Ueda K., S.L. Morgan, A. Fox, J. Gilbart, A. Sonesson, L. Larsson, and G. Odham, "D-Alanine as a Chemical Marker for Determination of Streptococcal Cell Wall Levels in Mammalian Tissues by Gas Chromatography Negative Ion/Chemical Ionization Mass Spectrometry," *Analyt. Chem.* **61**:265-270 (1989).

Wunschel, D.S., K.F. Fox, A. Fox, J.E. Bruce, D.C. Muddiman, and R.D. Smith, "Analysis of Double Stranded Polymerase Chain Reaction Products from the Bacillus Cereus Group by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry," *Rapid Commun. Mass Spectrom.* **10**:29-35 (1996).

Wunschel, D., K. Fox, A. Fox, M. Nagpal, K. Kim, G. Stewart, "Quantitative Analysis of Neutral and Acidic Sugars in Whole Bacterial Cell Hydrolysates Using High Performance Anion Exchange Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry," *J. Chromatogr.* (in press 1997).

Modern Techniques for Testing Pathogenicity and Hazards, with Special Reference to Humans

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Humans can become infected by various routes, including inhalation or ingestion of microorganisms, fecal-oral or venereal transmission, or contact with infected vectors or animals. The outcome of the interaction between humans and microorganisms depends primarily on the innate virulence of the microorganism and resistance of the host. Obviously, not every encounter results in disease. The successful pathogen is able to attach and enter into the body; can spread, locally or to all tissues and organs; can replicate inside the host; is able to evade host defenses; may damage the host; and is shed (exits) from the body, to ensure an encounter with another susceptible host. Recent research indicates that each of these steps is very complex and involves numerous microbial genes and gene products and physiologic processes of host cells.

Many pathogenic microorganisms possess specific adhesin molecules on their surface, that serve as ligands for attaching to host cell receptors. The nature and composition of the adhesins vary among different types of microorganisms, but adhesins of similar microorganisms have common features. For example, bacteria possess hair-like appendages (fimbriae or pili) on their outer surface, and the tips of the pili bind selectively to certain receptors on mammalian cells. In contrast, the hemagglutinin molecules of influenza and measles viruses, which comprise a major portion of the outer surface of these viruses, attach preferentially to the respiratory epithelium of host mammals. Regardless of the mechanism of attachment, normal host cell functions are subsequently re-directed to purposes that serve the invading microorganism. For example, attached bacteria produce and secrete a variety of effector molecules that direct the host cells to internalize the bacteria. Then, other bacterial molecules cause the host cells to produce strands of fibers that direct the movement of bacteria within and between host cells. To ensure their survival within the host, some pathogenic bacteria also produce molecules that cause the host to produce ineffective antibodies or to completely inhibit the immune response. Other microorganisms resist the immune response by producing different proteins on their outer surface while they multiply within a host (antigenic variation), such that antibodies are never able to neutralize the microbe.

Microorganisms damage cells and tissues in several ways: either directly by producing toxic byproducts; by inducing programmed cell death; or by the action of various toxins. The mechanisms of action of some bacterial toxins have been described at the molecular level. For example, adenylate cyclase toxin of the bacterium *Bordatella pertussis* has a molecular mass of 200,000. It is inactive until *B. pertussis* invades a host cell. However, once inside the host cell it becomes activated by a host cell enzyme and produces enormous levels of the physiologic

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modulator cyclic AMP. The elevated levels of cyclic AMP in turn disrupt several components of the host's immune response. Cholera toxin also increases cyclic AMP within gut tissue; however, in cholera infection, the cyclic AMP causes the cells lining the gut to discharge enormous quantities of water into the bowel, which is manifest clinically as profuse, watery diarrhea. Other microorganisms have different mechanisms of pathogenesis.

Most successful pathogens have evolved in concert with humans or other mammals and have learned to exploit host cell metabolic pathways. Not only do pathogenic microorganisms require organic compounds for their survival, many pathogens have very specific nutritional and metabolic requirements that can only be provided by host cells. In fact, certain microorganisms, such as rickettsiae and viruses, are obligate intracellular parasites that can only survive inside living cells. Thus, it seems unlikely that a microorganism that has evolved independently from prospective mammalian hosts would possess an innate pathogenic potential for humans.

In recent years molecular techniques have been developed to detect, identify, and differentiate various pathogenic microorganisms. Use of DNA probes, genomic sequencing, restriction fragment length polymorphisms (RFLP), Southern blotting, ribotyping, and multi-locus enzyme electrophoresis are examples. These techniques vary somewhat in sensitivity, specificity, and utility for identifying microorganisms. However, with certain exceptions, most existing DNA probes are used to detect "housekeeping" or cryptic genes and cannot detect specific virulence factors in microorganisms. The complexity of the pathogenic process precludes ready identification of a single gene or group of genes that can be considered generic markers of virulence.

Enteric bacteria are notable exceptions to this rule. The genomes of enteric bacteria possess large clusters of genes that contribute to a particular virulence phenotype. These "pathogenicity islands" were originally thought to be associated only with plasmids because they are transmissible between certain species of enteric bacteria; however, they have now been found on the bacterial chromosome as well. For example, the SPI-1 pathogenicity island of *Salmonella* contains at least 25 genes, which encode a secretion system and effector proteins that enable *Salmonella* to invade epithelial cells. A second island (SPI-2) contains at least 15 genes that ensure survival of *Salmonella* within host immune cells (macrophages). Pathogenicity islands are transferred among enteric bacteria by bacteriophage.

In summary, although pathogenesis is the subject of intense research, the molecular mechanisms remain incompletely understood for most microorganisms. Additionally, those pathogenic processes that have been determined are quite complex. Thus, functional assays remain the most reliable indicator of the pathogenic potential of a novel microorganism.

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SUBGROUP REPORTS

Containment Subgroup[2]

The Containment Subgroup discussed the development of recommendations that might be adopted by NASA for the safely controlled management of a Mars sample while a quarantine protocol is executed. "Containment" was defined as:

A system for protection of: a) the Earth's biosphere from release of "biological entities" of martian origin, and b) the integrity of the sample.

Sample Return Canister Considerations

It is essential that the entire system of containment prevent the escape of potentially hazardous material. Thus, consideration must be given to certain elements of the design of the sample return canister and the Earth return procedures. Decontamination of the exterior of the canister that contacts the martian surface must be a part of mission design. In addition, contingencies for non-nominal events should be pursued and incorporated into the design of the canister and return procedure. For example, the Earth Return Vehicle will be initially targeted away from Earth with a maneuver to place it on an Earth-entry trajectory. This maneuver will be executed following confirmation that the sample containment is intact. Following execution of that maneuver and Earth entry, should a breach occur, a mission design contingency should be provided for a suitable sample sterilization process.

A breach of canister integrity could also occur as a result of an unexpectedly hard impact at the landing site. Some provision should be made to determine if a breach occurs, and, again, a contingency planned for suitable sterilization in that event.

Upon recovery of the canister and re-confirmation that the sample has been properly contained, it will be necessary to transport the sample to a quarantine facility. This transit must meet regulatory requirements for safe transport of potentially hazardous biological material. These requirements include those of the U.S. Department of Transportation and the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS). The U.S. Public Health Service and some state agencies may also impose requirements for safe transport of the sample.

Precautions for handling the sample return canister until it has been sealed in a transport container should include the provision of protective garments for the recovery crews. These garments should meet or exceed EPA requirements for the clean-up of hazardous spills.

Contingency plans should also be developed for the impact site if an indicator shows that a breach of containment has occurred. Plans should be coordinated with APHIS and the U.S. Environmental Protection Agency to meet the regulatory requirements of those Agencies.

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2   The Containment Subgroup participants included: Mark Adler, Judy Allton, John Bagby (Chair), Jack Barengoltz, Ben Clark, Robert Hawley, Tullis Onstott, John Payne, Jonathan Richmond, and Perry Stabekis.

The Mars Receiving Laboratory

General Considerations: Decisions regarding the nature of containment to be accorded Mars samples were reached after considerable discussion. It was agreed that the unknown nature of any possible hazardous material in the sample demands provision of the most stringent containment presently afforded to the most hazardous biological entities known on Earth; that is, a Biosafety Level 4 (BSL-4) operation. Appropriate containment is attained through the application of primary and secondary containment principles.

Primary containment will be provided by utilizing Class III biosafety cabinets: glove boxes connected in sequence with sealable doors between cabinets and maintained under negative pressure.

Secondary containment is provided by the building: a "high-end" BSL-3 structure which is sealed and maintained under negative pressure, with high efficiency particulate air (HEPA)-filtered exhaust air, sterilized waste water, and with provisions for personnel showers and appropriate use of disinfectants.

Several laboratories do exist that provide BSL-4 protection required for the Mars samples. These labs have the added advantages of highly competent professional and support staff who are trained and experienced in the laboratory procedures with which they will work, and who have access to the necessary instrumentation.

Alternative Approaches: Biological safety and physical security must be the prime consideration in the design of a Mars Receiving Laboratory (MRL). The Subgroup considered alternate approaches to the provision of containment and security that included consideration of cost and efficiency.

The MRL must be constructed to meet a wide range of specifications. The recommended containment approach (Class III biological safety cabinets sealed sequentially) is flexible and portable and may be engineered to any size required for quarantine testing. If quarantine testing is limited to a few procedures in the initial phase, one cost effective option would be to provide a small MRL facility adjacent to an existing BSL-4 laboratory, e.g., USAMRIID at Fort Detrick, Maryland or the CDC laboratory in Atlanta, Georgia. If it becomes necessary to examine the samples beyond initial testing, the samples could be transferred to the BSL-4 laboratory without unnecessary transport. The more important advantage is the proximate availability of highly skilled, trained professionals to support staff who are experienced with total containment procedures. The Fort Detrick military facility also has the advantage of the Foreign Disease Weed Research Laboratory, a BSL-3 plus facility which is recognized by the Dept. of Agriculture for the containment and examination of non-indigenous plant pathogens.

Construction of a small facility as an adjunct to an existing approved facility should simplify approval of an Environmental Impact Statement (EIS) because the new elements in the EIS could be tiered to an existing analysis previously approved for the existing facility.

A potential disadvantage with an existing facility as back-up is the possible reduction of control of the samples by NASA while in the hands of another agency. However, existing policy for the transport and receipt by a facility of potentially hazardous agents requires CDC to review the facility (e.g., the USAMRIID laboratory), thus providing an additional check on safety. Other interagency agreements could be negotiated.

Existing containment facilities usually have no restrictions on organic contamination of the samples. Because of the requirement for an environment free of organic molecules for the Mars samples, a process change in the operation of primary containment devices in existing facilities will be necessary in order to comply. Facility management issues are very critical to the entire operation regardless of facility location, and require early study, negotiation, and resolution.

If a decision is made to provide a dedicated new facility to receive, contain, and examine Mars samples, that decision needs to be made soon. One of the most serious problems faced by the Apollo Program's Lunar Receiving Laboratory was the time constraint imposed on that laboratory by the extreme timetable for the Apollo Moon missions and the delay in planning for planetary protection. At least five years must be allowed for the construction and certification of such a highly technical facility and for the training of professional and support staff. Training periods are required for qualified personnel to become familiar with a new facility so that their operations are safe, efficient, and accurate.

The Glove Box System: Flexibility of the glove box system will allow design and construction to meet a broad range of requirements established by the quarantine protocol. Glove boxes can be designed to include any laboratory equipment required by the protocols as well. Operational parts of the equipment can be housed within the primary containment glove boxes, with the electronics, control panels, etc., located outside the primary containment barrier.

Both the inside and outside surfaces of the box used to transport the sample canister from the landing site to the receiving facility will be contaminated with Earth organisms during the process of depositing the sample canister inside. When the transport box is inserted into the first Class III biological safety cabinet, that cabinet will also become contaminated. The canister itself should be removed from the box and moved through an airlock into a second Class III biological safety cabinet, then the first cabinet is sealed off. If the canister has been breached, then the transport box remains in quarantine, or is sterilized, after opening it in the first cabinet.

In the second cabinet, a gas sample could be removed from the canister head space if the canister is not breached. The canister would then be attached to a specially fitted gate that separates the second cabinet from the third. This gate will permit the canister to be opened from the third cabinet, its contents removed to the third cabinet and the canister itself left in the second cabinet. Thus, the third cabinet contains the Mars samples that are completely separated from contamination picked up during recovery and transit. In the third cabinet samples may be examined and decisions made concerning portions to be used for quarantine testing and portions to be archived for future analysis. The archive sample should be placed in a sterile container under positive pressure relative to the third cabinet. Storage temperature and atmosphere should be approximately Mars-equivalent.

The next cabinet and additional ones linked in series for quarantine testing will be maintained under negative pressure to satisfy primary containment requirements for environmental protection and worker safety. The building itself is constructed and operated to provide a secondary barrier.

The Class III biological safety cabinets will be sterilized for terrestrial organisms until quarantine testing reaches those cabinets where plant and animal hazard testing may be required. Only inorganic sterilants without carbon residue should be used.

The deployment of canister and samples through the boxes will depend on what tests are to be run and at what stage of the operation. For example, analytic equipment for sample manipulation might fill one cabinet and leave no room for other operations. The flexible cabinet system will allow for any series of operations to be designed and conducted.

Summary

The Containment Subgroup considered requirements for containment of the anticipated Mars material that may reach Earth in 2008. Integrity of the sample container upon entry into the Earth's biosphere and verification of containment were recognized as important and recommendations are made in this report for certain aspects of container design and recovery techniques.

Once the sealed, un-breached canister has been recovered and safely transported to an MRL it should be placed in BSL-4 containment attained by a double biological barrier. Primary containment is to be provided with Class III cabinets maintained under negative pressure to the room in which they are installed. Additional containment is provided by a "high end" BSL-3 building. There the transport box and the canister will be opened and a sample will be removed for quarantine testing. The remaining sample is then sealed and maintained under positive pressure.

The quarantine portion of the sample is moved into a connecting cabinet/glove box under negative pressure. It will be examined under quarantine protocol requirements. The Class III biological safety cabinet system has flexibility that will allow any number of cabinets to be connected in series, joined with sealable doors and include any equipment required for analysis.

Cost and efficiency considerations indicate that a small initial MRL might be built in close proximity to one of the existing BSL-4 biocontainment laboratories in the United States. The MRL will perform tests to insure the samples are safe to release for scientific investigations. If any indication of biological activity is detected during quarantine testing, the samples could be moved quickly and safely into the adjacent BSL-4 facility for further analysis (e.g., toxicity/pathogenicity).

Whether decisions lead to the design and construction of a small, initial MRL or a full-scale facility dedicated to initial and follow-up testing, action should begin soon to allow adequate time for design, construction, and certification of the laboratory, as well as recruitment and training of the professional and support staff.

<u>Research and Technology Needs</u>

Recommendations for research that will be required for successful containment of Mars samples both while in transit and in the laboratory on Earth are provided here:

1. HEPA filters will be required for the MRL and can be designed for the Sample Return Canister (SRC). These filters can be arranged in series to increase their efficiency. Such filtration is recognized as the proven standard for high maximum containment of the most hazardous biological agents known. The Subgroup recommends that challenge testing of the filtration systems be undertaken using carbon-bearing particles from 10 nm to 100 nm in size.

2. For in-flight verification of the canister seals it would be inappropriate to use carbon compounds. To avoid sample contamination it may be desirable to use radioactive-tagged particles. Research should be conducted to choose appropriate isotopes and particle sizes for such verification/testing.

3. Research is required to select an appropriate indicator for canister seal integrity upon recovery.

4. It will be necessary to design effective processes to clean the containment area of terrestrial biological entities and organics to avoid confusion during observations of the Mars samples.

5. Systems must be developed and tested to maintain sample integrity when obtaining aliquots of material for quarantine testing.

6. Canister design research could provide a system for needle puncture of the "head space" through a vacuum-sealed line; HEPA filters could be incorporated.

7. Research should be undertaken to determine suitable sterilization methods for the Mars sample.

Life Detection Subgroup[3]

<u>Background</u>

The capability to detect life defines a root issue in planetary protection. If there is no life, there is no biological hazard. The presence of live organisms in a returned sample suggests terrestrial contamination or the presence of a possibly hazardous (although certainly quite interesting) example of extraterrestrial biology. The Space Studies Board has made recommendations which stress improvements in life detection techniques in both their report on the Biological Contamination of Mars [Space Studies Board 1992], and in their recent report on Mars Sample Return [Space Studies Board 1997]. In the former, life detection techniques employed to gauge the level of contamination on outbound spacecraft were emphasized, while in the latter report the

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3 The Life Detection Subgroup participants included: Carlton Allen, Don DeVincenzi, Jack Farmer, Alvin Fox, Harold P. Klein, Harold Morowitz, Norman Pace, Tommy J. Phelps, John D. Rummel (Chair), and Norman Wainwright.

emphasis was on the detection of live organisms and to differentiate between organisms from Earth and those that may be found on Mars. From the planetary protection standpoint, the emphasis for sample return missions is to ascertain the potential for extraterrestrially-introduced hazards that may be carried in the martian sample.

We do not have a universal definition of life, and thus it must be said that any life detection protocol that is offered for the testing of extraterrestrial samples will have certain Earth-oriented prejudices built in. This is unavoidable, but serves to limit the scope of the detection protocol to activities that would be consistent with the detection of Earth life, in its many forms. Developing a protocol that would routinely be successful in the detection of life, and in particular any live organisms, would, in and of itself, be a prodigious achievement.

Introduction

The Life Detection Subgroup was assigned the task to develop a series of tests (a protocol) to detect the presence of live organisms, or of materials that have been derived from live organisms, in a sample of material returned from Mars. In order to define these tests, the Subgroup considered the likely aspects of viable organisms that might be detected (irrespective of the origin of the living entities). The philosophy that should guide the life detection protocol, which in turn would dictate the sequence, techniques, and handling requirements for the protocol was also defined. In addition, the Subgroup made recommendations on research that might be required to further refine the details of the tests to be made.

The philosophy that the Subgroup espoused during the Workshop was aimed not only at detecting life, but distinguishing between potential martian life forms and terrestrial contamination. The essence of the philosophy is twofold: 1) that there must be multiple lines of evidence to support an hypothesis that detected life is of martian origin; and 2) it is essential to understand the geological and potential ecological context of a sample in order to understand the nature of life that might be detected in that sample. In support of this philosophy, it was deemed essential to have a strong quality assurance (QA)/quality control (QC) program, involving the use of chemical tracers, as part of a sample return mission in order to correlate the "detected" material/organism(s) with the phase of the spaceflight mission in which that material was obtained.

In order to establish the appropriate context for life detection in a sample, the Subgroup recommended that a preliminary analysis of the sample be conducted. The analysis should: 1) characterize the bulk mineralogy of the sample, 2) establish its elemental composition, 3) inventory the volatile and organic materials it may contain, 4) measure the redox couples present in the sample material, and 5) obtain a microscopic characterization of the sample's surface and interior. It was felt that most of these analyses would not require that a sample be held in biological containment, as long as an adequate sterilization method could be defined which would not affect the results of the analysis.

The Subgroup prioritized three basic methods by which life detection could be accomplished:

1. Organic chemical analysis and detection: These would offer the most compelling probability of detecting the presence of living organisms in a sample. This analysis would include a

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search for functional groups containing reduced carbon, sulfur or nitrogen; an analysis of possible kerogen materials for stable isotope abundances; the detection of amino acids or possible proteins; an analysis for amphiphiles in the form of fatty acids, hopanes, etc.; a search for carbohydrates, nucleic acid bases, and related compounds such as DNA, RNA, PNA, etc.; and the potential detection of integrated cell walls or cell wall components such as lipopolysaccharides. With current improvements in the technologies available for these analyses, cellular life could be detected routinely at the level of 10-100 cells in a sample, and potentially these techniques could be successful in detecting as little as one cell in a 100 g sample.

2. Light and/or electron microscopy: This technique would be used to detect the morphological indications of life, along with the trace mineralogy of the sample. Coupled with staining methods that can reveal chemical evidence of life in conjunction with the morphological methods, light microscopy has a number of advantages over electron microscopy in terms of sample preparation and handling, and in real-time testing of a sample. Nonetheless, electron microscopy, and particularly ion-probe techniques, can provide critical information about a given sample in terms of composition. Microscopic techniques provide the potential to detect as little as one cell in a given sample; however, what constitutes a "representative" sample would need to be defined.

3. The culturing of martian materials and/or living organisms: This is much more difficult to generalize for putative martian organisms, though it provides a natural link to other planetary protection activities such as hazard detection. Nonetheless, cultivation as a life detection approach was recommended for adoption because of the potential to amplify the presence of life in a sample (through growth and reproduction). It may be the most effective means by which to discriminate between a viable organism and materials that were once associated with biology, but are not now alive. Cultivation techniques to be attempted should include not only conditions commensurate with the environment from which the samples were obtained, but also the use of multiple media and carbon sources under both aerobic and anaerobic conditions, using both intact samples and processed sample materials.

A Life Detection Protocol

The processing of martian samples for life detection purposes should be an integrated facet of a comprehensive analysis of the samples for atmospheric, geophysical, and exobiological purposes. The specific steps to be taken in the early phases of sample handling and preparation would be driven by the nature of the specific samples collected on Mars. It is clear that gases, dust/soil, and rocks will each present specialized sample canister, containment, and analysis challenges. Despite these variations, a comprehensive process for sample analysis and life detection follows:

Sample Container: The nature of the sample container and the process for collection of samples on Mars remains to be determined, and clearly will affect the subsequent analysis on Earth. For example, it would be useful to have a good unmixed sample of the martian atmosphere from the sample location to compare with headspace gases in the sample chamber(s) once the sample container is returned to Earth. It would be desirable to be able to sequester rock samples,

particularly, and other samples taken from below the surface of a rock from the martian dust/soil material that will also be sampled. This segregation will be useful for later analysis and sample handling on Earth.

Equally important will be to characterize the physical (and chemical) conditions of the sample(s) during their transit from the surface of Mars to the receiving laboratory on Earth. Proper instrumentation in the sample container should be provided to record the temperatures and pressures experienced by the sample(s) during the transit and recovery process.

Sample Receiving: The sample receiving process has several goals. Care must be taken to avoid sample alteration during the transfer of the sample from the container. Alteration could destroy critical evidence about the state of the sample when it was on Mars. Another prime goal during sample handling, for the purposes of a life detection protocol, is to avoid the introduction of Earth organisms or other contaminants into the sample that might provide a false positive indication during life detection testing. Other concerns (i.e., the inadvertent release of material in an uncontrolled fashion), are inherent in handling potentially biohazardous materials. But it is important to note that there may be a conflict between the desire to keep the sample pristine and some measures required to prevent the escape of putative organisms from the sample.

Sample Separation: Prior to removal of a sample from the container, headspace gas should be analyzed for evolved gases. This provides an indication of gas absorption or desorption by the sample and will enable other comparisons to the ambient martian atmospheric sample. Such analysis can provide important insights into the nature of the sample chemistry during the trip from Mars.

At each step in the process of sample separation and analysis, it is important that multiple samples be allocated for each test and be photographed and weighed. In addition, some samples must be preserved for archival purposes. It should be emphasized that later test results may require procedures used in the initial work to be repeated, to some degree, or may dictate alternative testing techniques that were not initially envisioned.

After sampling the headspace gases and separating any atmospheric sample that may have been returned with the sample canister, the next step in sample processing would be to remove the solid sample from its container, and separate dust and soil samples from rock samples collected on Mars. The Subgroup strongly favored that the sample return mission accomplish the bulk separation of these materials as they are collected. A failure to do so will greatly complicate the sample separation process, and could result in the loss of critical data about each sample's provenance, or serve to make such data impossible to interpret. The Subgroup noted that an understanding of the sample's context on Mars is essential to the ability to correctly interpret chemical analyses on Earth. Also, the return of a sample in bulk is inimical to an effective QA/QC program whose purpose is to aid in identifying the source of any contamination that may be introduced into a sample and/or sample chamber during collection and retrieval missions.

Microscopic/Mineralogical/Geochemical Survey: Reflected light microscopy at low power is considered the most logical initial screening technique to group samples for more detailed analysis. Along with the use of a micro-photometer, samples can be analyzed to determine their

petrography, mineralogy, and state of alteration. It may also be possible to compare the analysis of martian samples on Earth with *in situ* analysis of the sample on Mars. This will indicate any changes in composition during transit to Earth.

Rock samples can be broken to expose their interiors, and representative sub-samples can be selected from each rock for further life detection analyses, as well as for subsequent curation and distribution. The rock and dust sub-samples could be subjected to a certified sterilization process and distributed outside of the containment facility for special-purpose geological and geochemical analysis by external laboratories. Many appropriate geoscience analyses, however, may be compromised by heat and steam, and may have to be conducted within the containment facility, or await the release of the sample. Among the analyses that could be conducted at this point are the establishment of the general elemental, isotopic, and chemical composition of the sample, an assay for paleomagnetism, and isotope dating of the sample.

Life Detection Microscopy: Microscopy will be essential for the initial screening of martian samples, and for certain geological analyses. There are a number of techniques in microscopy that will be particularly advantageous to employ in specific areas of life detection. Some of these techniques will be a continuation and intensification of those used in the screening process. A petrofabrics analysis of the rock sections by light microscopy (transmitted light, polarized light, UV fluorescence, IR reflected cathodoluminescence) would be to determine mineralogy, and to search for biologically-induced structures or fossils preserved by mineral replacement which would not be apparent through organic chemical analysis.

Biological applications of microscopy might involve staining of samples using, for example, DNA probes or other biostains and fluorescence techniques that would allow for the visualization of biomaterials, if present. For transparent minerals, a fluorescent stain method with confocal microscopy could document the distribution of any organic materials present, down to the size range of light microscopy (in the range of a few micrometers).

Wavelength-specific x-ray imaging using a synchrotron facility, with similar resolution to light microscopy, can provide detailed information about the redox states of elements like iron, manganese, and other important metals of potential biological importance. While it would likely have to be applied outside of containment, this method also permits the detection of specific types of organic molecules and minerals and their spatial distribution from the x-ray images and tomography.

For high-resolution imaging, electron microscopy offers a wide range of potential techniques for elemental, mineralogical, and biological analysis. To characterize the mineralogical/geochemical and petrofabrics framework of samples, scanning electron microscopy (SEM) is an important tool. SEM provides an initial visualization of microfabrics of mineralized frameworks linking light microscopy with transmission electron microscopy (TEM). The utility of SEM is that it involves little destruction of material, and perhaps only a thin coating of conductive material to prevent a charge on the sample. Chrome or iridium should be used for coating or, where possible, uncoated samples should be examined to avoid introducing artifacts caused by the coating process that could lead to misinterpretation of results.

SEM can also be used in back-scatter mode, to visualize spatial variations in atomic number, with an Energy Dispersive X-Ray Detector (EDX) to produce maps of major elemental distributions. To link these analyses to more sensitive microprobe methods, SEM for petrofabrics can also be carried out on lightly etched thin sections. Detecting biological microstructures (e.g., cellular remains) by SEM may involve the application of biological fixatives and critical point drying methods to samples to ensure the preservation of organic materials. This can be done without interfering with mineralogical analyses, providing biomaterials are scarce in the sample.

TEM can provide important information on ultrastructure and mineralogy that is needed to fully describe the sample. If biologically interesting structures are imaged, a logical next step would be to prepare those samples for TEM to obtain cross-sectional views and to further characterize their microstructure and mineralogy at higher resolution. TEM methods require sub-sectioning of targeted domains into thin sections either by ultramicrotomy, which typically destroys spatial frameworks in mineralized frameworks, or by ion milling of sections. Precision sectioning could also be accomplished within an SEM – a method newly developed by Hitachi.

Selected staining of biological materials for TEM provides a means for attaining a detailed characterization of the ultrastructure and general composition of biological materials present. The challenge of ion-milling techniques center around the preferential thinning and loss of organic components during sample preparation.

Thin-section based approaches can be coupled to laser-based mass spectrometry methods to determine the isotopic composition of minerals and organics present in rocks, or to related high resolution techniques (e.g., ion probe) for carrying out organic analysis on rock samples. These techniques can determine spatial distribution of key minerals, elements, and organic compounds.

Chemical Analyses for Signs of Life: The Subgroup agreed that the highest priority life detection investigations should be comprised of organic chemical analysis and detection – a search for functional groups. These analyses can be used to detect life, or life-related molecules, at a level of detection approaching, and in some cases exceeding, that required to detect a single cell.

Assuming a terrestrial bacteria with $\sim$1 μm^3 volume, a cell mass of $\sim$0.6 picograms, a cellular biomass based on chromate oxidation, and a mole molecular weight of 104 g, each hydrolyzed cell would have greater than 10^9 organic molecules with a molecular weight of $\sim$100, after accounting for the water in the volumetric approach. With infrared micro-calorimetric detection for amine groups and again for carboxyl groups there are dual independent measures capable of detecting as few as 10,000 such molecules. This detection level provides five orders of magnitude of surplus margin that may be important in detection of cells that may be smaller than those found on Earth. This also allows for mismatches in detection systems, and/or upscaling problems. This level of detectability for critical components of terrestrial living systems is available.

There are, of course, caveats associated with the use of chemical detection alone. The Subgroup has recommended that confirmation of chemical tests be accompanied by other evidence for life, such as the demonstration of metabolic function by living systems. The dependence on organic chemical detection alone means it is nearly impossible to differentiate between traces of living and

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dead organisms. In fact, it may be easier to identify the kind, or form, of an organism than it is to show its function.

Several techniques and targets for organic chemical analysis and detection were discussed by the Subgroup:

1. Mass Spectrometry Methods: It may be a reasonable first approximation to assume that life, if present on Mars, is based on the biogenic elements and common terrestrial organic compounds such as amino acids, sugars, fatty acids, and nucleotides polymerized respectively into proteins, polysaccharides, lipids and nucleic acids. While it is possible these martian compounds may be identical to terrestrial compounds it is also possible that these compounds may be entirely distinct from those present in terrestrial organisms. Thus, the analytical methods selected should be of general applicability and capable of profiling the whole class of each type of compound (e.g., L- and D-amino acids) potentially associated with martian life.

As discussed earlier (see pg. 17) the Viking missions to Mars in the 1970s utilized pyrolysis mass spectrometry as an analytical technique. Problems inherent in this technique were later discovered and from the late 1970s into the 1990s, techniques based on "derivatization and mass spectrometry" were successfully developed for detection of trace amounts of chemical markers for bacteria in complex clinical and environmental matrices. Most recently, "tandem" techniques such as GC-MS and GC-MS-MS have resulted in further improvements for detection and identification of trace native molecules in complex environmental samples, however, trace detection of microbial components in complex environmental matrices remains a novel, high-technology research area (see page 18 for examples and further discussion). Techniques such as electrospray ionization or matrix-assisted laser desorption are currently under development and have the potential for detection of trace amounts of polar and/or high molecular weight chemical markers for bacteria (e.g., nucleic acids, proteins and phospholipids) in complex matrices with minimal sample preparation.

2. Combined PCR/Mass Spectrometry: This combination, developed in a collaboration between PNL (Pacific Northwest National Laboratory) and the University of South Carolina, provides an example of the use of electrospray ionization for chemical analysis of bacterial nucleic acids. The combined technique provides a rapid (<10 min) method to identify pathogenic bacteria (see pg. 19 for further detail).

This technique might be particularly important for the detection of terrestrial contamination in a returned sample from Mars. Because the use of PCR assumes that the organisms involved will have nucleic acids like those found in Earth organisms, the detection of specific genetic regions from organisms potentially in martian rock is premature, at best, and would not be recommended at this time. Nonetheless, this approach is important because it demonstrates the ability to detect intact high molecular weight oligomers and polymers derived from bacteria with minimal sample work-up and no derivatization.

3. Detection of Amphiphiles: Amphiphiles are molecules with both polar and non-polar portions, and include fatty acids, sterols, and hopanoids. In aqueous media they are the basis of

coacervate formation, including biomolecular leaflets, membranes, and vesicles. They are a *sine qua non* of cellularity. Amphiphiles can be detected by extraction of ground rock with chloroform and methanol (2:1), followed by drying of the supernatant. Amphiphiles can then be detected by suspending the dried material in an aqueous phase and sonicating (structural disruption with sound waves). The sonicate can then be studied by light and electron microscopy.

Amphiphiles have been reported in some carbonaceous chondrites [cf., Deamer 1997]. There is a preponderance of cyclic aliphatic hydrocarbons in extraterrestrial materials currently available. The presence of long-chain aliphatic hydrocarbons in a martian sample would be of great interest to chemists studying prebiotic chemical evolution and to scientists concerned with life detection techniques.

4. Cell Wall Components: Another family of target compounds indicative of living systems includes materials that make up the cell walls of free-living microorganisms on Earth, i.e., lipopolysaccharides (LPS), beta glycans, and peptidoglycans. Current methods for the detection of these compounds use an amplification system based on the primitive anti-microbial defense system found in the blood of the horeseshoe crab, *Limulus polyphemus*, and other marine and terrestrial invertebrates. The current level of detection is 100 femtograms and it is likely that this level of detection could be improved by at least an order of magnitude over the next several years with the appropriate research and development. Cell wall materials are generally robust chemical compounds that could survive some sterilization procedures (autoclaving) but would be unlikely to survive some other methods (e.g., dry heat at 200 °C for several hours).

Summary of the Chemical Analytical Approach: The following considerations form the basic concept of chemical analysis techniques in life detection:

1. Seek functional groups important for energy transfer rather than live biomass (look at many millions of molecules rather than a single cell).

2. Do not simply identify or profile cells, but seek to identify accumulated biomass-type molecules – lipids and RNA/DNA sequencing look at only a very small percentage of the components of biomass to identify a cell type, and consequently require hundreds of cells for detection. By looking at hydrolysis products, there is a gain of orders-of-magnitude in sensitivity, though at a complete loss of selectivity. Looking at cellular components can enable the detection of materials at the level of 1% of those required for a single living entity.

3. Use more sensitive and less selective detectors for the first sample screening procedure. Rather than employing the selectivity of GC-MS or LC-MS (liquid chromatography-mass spectrometry) as the first step, highly sensitive infrared micro-calorimetric or lab-on-a-chip technology can be employed to provide high sensitivity detection of functional groups.

4. Integrate remnant parts. On Earth, the amount of functional groups remaining from remnant parts often exceeds the live biomass. These materials, if present, will not of themselves provide an indication of the extant life. However, a more selective analysis might be

undertaken to detect extant life that may be related to the organisms that produced the non-living materials. Paradoxically, the oxidized conditions on Mars make it likely that unprotected functional groups in materials on the surface would have a relatively short duration (less than centuries). This would suggest that accumulation of carboxyl and amine functional groups could represent organized and somewhat recent production; which may indicate the presence of life.

5. Because extraterrestrial life may be markedly different in detail from life on Earth, it may not be possible to rely on DNA, RNA, proteins, or even carbon-based molecular backbones as indicators of life. By focusing initial screening efforts on amine and carboxyl functional groups it may be possible to detect signs of life based on any backbone, C, N, P, S, or Si. It is possible that samples may contain non-life related organics such as PAHs or benzenes that are ill-equipped for high energy electron transfers required for the electron accepting/donating/transfer processes essential for life. Comparison of stable isotopic signatures of non-life-like compounds (e.g., PAHs) and life-like compounds can be made using GC-Isotope Ratio -MS, and may provide additional information on the potential existence of life on Mars.

Biological Activity/Biohazard Testing: The technique of bacterial culturing is a powerful and simple approach for the detection of life that may be employed in studies of putative martian life, provided the nutritional requirements of such life are known. Well-known causative agents of human disease, that have been studied for many decades, cannot be cultured on conventional microbiological media. Indeed, in many instances they do not grow on any currently available laboratory media. Thus, even if a sample will not grow in culture, it still may contain life. This limitation makes culturing of microbes of less importance to life detection than to the area of biohazard testing, where the lack of positive organismal growth in some instances may be considered sufficient to declare a sample non-hazardous.

Given that the culturability of environmental microbes from Earth is very low (<1%), there is an extremely low probability of achieving the appropriate media conditions for the propagation of martian microbes. Culturability is thus of secondary or tertiary priority for life detection. Nonetheless, the methods are simple enough to consider rudimentary experiments to attempt growth in culture. These attempts will be complementary to biohazard testing that may be required. The possibility does exist that extraterrestrial microbes could subsist as spores or in a dormant state. Theoretically, positive growth detection would be more sensitive than many of the physical methods for the detection of biogenic compounds. A single viable cell would be the only initial requirement. By definition, test samples would not survive sterilization.

The geological aspects of the sample site(s) could affect likely culturing conditions. For example, attempts to culture samples taken in an area of possible volcanic activity may suggest including in the medium possible energy sources such as hydrogen sulfide. For samples from deep cores, or from sedimentary rock or impact ejecta, it may be possible to simulate specific conditions that would mimic the micro-environments from which the samples were collected.

Perhaps the greatest obstacle to the existence of life on Mars is the absence of liquid water, with perhaps two orders of magnitude less than the driest environments on Earth. Therefore,

specialized mechanisms of subterranean water capture or desiccation-tolerant organisms may require culture conditions that provide for transient or miniscule water applications.

If more conventional media formulations are used, a matrix of nutrients in a microtiter plate format can be used to minimize sample requirements, and maximize the range of nutrient concentrations tested. Cultures with sample material should be tested within the temperature and pressure conditions that include those of the present and near-past martian environmental range.

Quality Assurance/Quality Control

Throughout the mission and during the analysis of samples for the purposes of life detection, an emphasis on quality assurance and quality control (QA/QC) will have to be maintained. Extensive QA/QC activities are, in fact, required to determine that the evidence of life is not from Earthly contamination. The use of marker agents and tracer compounds to correlate sample contents with mission phases should be envisioned as an integral part of the sample return process. Similar activities have been essential to our understanding of the microbial world on Earth, and in particular to the Department of Energy's Subsurface Science Program.

Research and Technology Needs

The missions to return rock samples from Mars to Earth will begin early in the next century and procedures for containment and determination of biohazard of returned samples are under development. However, most techniques to determine biohazard of samples are based on traditional microbiological culturing techniques and have not been developed for the study of possible extraterrestrial life. NASA must begin to incorporate life detection technologies into the planning and anticipated sample receiving activities for the return of martian samples. For example, a plan must be developed for the acquisition and operation of the appropriate instrumentation within the sample handling facility. Additionally, a protocol for appropriate sterilization methods must be initiated to prepare samples for distribution to the wider scientific community. Such a protocol must be developed in a collaboration between exobiologists and those skilled in the area of analytical microbiology.

References

Deamer, D.W., "The First Living Systems: A Bioenergetic Perspective," *Microbiology and Molecular Biology Reviews* **61**:239-261 (1997).

Space Studies Board, National Research Council, *Biological Contamination of Mars: Issues and Recommendations*, National Academy Press, Washington, D.C. (1992).

Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C. (1997).

Biohazard and Testing Subgroup[4]

<u>Background</u>

With the prospect of a Mars sample return mission as early as 2005, it is appropriate to consider how to ensure that materials deliberately returned from another planet or solar system body will have no adverse effect on the Earth's biosphere. 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, within rocks or in subsurface regions. Based on evidence from studies of life on Earth, we now recognize that living organisms can possess unusual resiliency in the face of extreme environments, a tenacious ability to survive over long times, and wide versatility with respect to sources of energy utilized [Nealson 1997]. Thus, although it is highly improbable that life could be found in collected martian surface material, it does remain possible.

In their recent report on Mars Sample Return, the Space Studies Board noted that while contamination of Earth by putative martian microorganisms is unlikely to pose a risk of significant ecological impact or other significant harmful effects, the risk is not zero [Space Studies Board 1997]. Accordingly, the report recommends a cautious approach combining strict sample containment, systematic analytical investigations, and an operational assumption that returned materials be considered hazardous to biology until tested and proven otherwise. To translate these recommendations into protocols that can then be implemented is clearly a complicated task.

Once martian samples are returned to an appropriate quarantine facility, the initial biological screening will fall into two, non-exclusive categories: 1) investigation for the presence and properties of biological entities in the samples, and 2) investigations of whether the samples pose any threat to terrestrial biology or ecology. Our scientific understanding and technical capabilities for both these tasks have improved enormously in the decades since the Apollo program when the first extraterrestrial materials were returned to Earth from the moon. Thus, as we undertake the task of developing preliminary Mars sample testing protocols, we have a broader perspective and considerably more information to build upon. However, since the knowledge and capabilities are based upon research with organisms from Earth, we recognize that some amount of judgment and extrapolation will also be required in areas of scientific uncertainty as protocols are developed.

<u>Introduction</u>

The Biohazard Testing Subgroup was assigned the task to develop an up-to-date methodology to determine if returned martian sample materials are hazardous, regardless of whether life or biological entities are detected. The Subgroup assumed that all biohazard screening would be conducted in conjunction with a systematic battery of life detection studies and chemical characterizations of sample materials. The group considered that biohazard protocols must anticipate the prospect that any biological entity in the samples is likely to be at very low levels of detection and may not be culturable.

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[4] Members of the Biohazard Subgroup included: Gerda Horneck, Daniel A. Kluepfel, Joseph McDade, Harold Morowitz, Ronald Oremland, James Pearson, Margaret Race (Chair), and Zigfridas Vaituzis.

Work began with a brief review of relevant biohazard protocols that were used in the Apollo Program [Allton 1997] and those proposed for martian samples in the Antaeus Report [DeVincenzi and Bagby 1981]. In addition, the comparative approaches to biohazard testing that are currently used by USDA, CDC, and EPA for various pathogens, etiologic agents, and environmental toxins were discussed. The Subgroup determined that it would be advisable to base a protocol upon the successful record and extensive data base of biohazard protocols currently used by researchers and agencies for a wide range of biological agents. Building on this information, a tiered or stepwise approach to testing was proposed. These tests would: 1) focus on a broad range of biohazards, 2) screen for indications of biological activity or disruption thereof, and 3) incorporate systematic feedback as data are gathered from the life detection studies, chemical analyses, and biohazard tests themselves. Operationally, the task was translated into the following two questions: What set of tests, at a minimum, would provide sufficient information to determine if controlled distribution of martian sample materials could be allowed outside strict biological containment? Additionally, what research and technology needs must be pursued in order to specify detailed procedures and contribute to successful implementation?

Development of a Biohazard Testing Protocol

A concentration on biologically relevant views of hazard would be useful to determine whether samples are safe to distribute in a controlled manner, initially within the receiving facility, and eventually outside of it. The use of the terms 'hazardous' or 'biohazard' deliberately avoided any reference to definitions that are linked to particular laws, regulations, or agencies based on the uniqueness of returning a sample of unknown biological potential.

Samples returned from Mars could be considered a biological hazard or environmental concern for several reasons: a) they could contain toxic materials and thereby pose a threat to investigators that work with the samples; b) they could contain entities that might be pathogenic for Earth organisms; or c) they could harbor organisms capable of thriving on the Earth and displacing native life forms. For reasons elaborated below, we placed our emphasis on hazards posed by organisms that replicate because of their potential for large scale negative impacts on the Earth's diverse ecosystem. Conceptually, the concerns are similar to those impacts routinely encountered on Earth in the areas of environment, health, and safety that are caused either by disease transmission agents, or by the transportation, importation, or invasion of non-native biological agents or organisms, whether natural or genetically engineered. The concerns are:

1. Chemical Toxicity: Because of the small amount of material to be returned, the concern about chemical toxicity was not considered a significant hazard or global threat since toxic materials will not replicate and spread. Presumably, any threat to investigators from martian soils or rocks would be revealed during the initial chemical analyses carried out under strict containment with current test protocols. Screening technology will be advanced by the time samples are returned in 2008, as will our understanding about the martian surface. In the event that chemical test results from future robotic one-way missions indicate an unusual concern about toxicity, the appropriate handling guidelines or investigations of the samples could be instituted, analogous to terrestrial geological samples of interest.

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2. Pathogenicity: As discussed in the SSB report [Space Studies Board 1997], pathogenesis can be divided into two fundamental types: toxic and infectious. Generally, biologically induced toxic effects of microorganisms are attributable to cell components or metabolic products that incidentally damage other organisms. Infectious agents, which may be actively or opportunistically invasive, must multiply in or on a host in order to cause damage. Both toxicity and infectivity are of concern for martian samples because they represent hazards posed by the presumed presence of potentially replicating organisms, however rare or hard to detect they may be in the sample. Regardless of the outcome of preliminary life detection tests or chemical analyses, it will be prudent to also screen samples for both types of pathogenicity with tests specifically designed to detect biological activity or disruptions.

During the Apollo program, evidence of the presence of pathogenic effects and infectious agents in samples relied on a combination of microbiological cultivation tests and experiments that challenged select whole animals and plants via exposure to sample material. The challenge test protocols initially included 69 species from ten animal phyla and 34 species in nine plant divisions [Allton 1997]. This required elaborate animal and plant support and contamination controls in association with the quarantine facility. The significant advances in the use of model systems and tissue culture, as well as improvements in technological capabilities over the past several decades, suggest that it would not be necessary to conduct whole organism challenge tests as part of the first line to screen for returned martian samples.

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

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

Detailed information is provided in Appendix A.1 on the various pathogenicity tests that were tentatively proposed and discussed.

3. Ecological Disruption: Based on our knowledge of martian surface conditions and terrestrial life, putative martian organisms may be functionally similar to microbes found in soil and rock habitats on Earth. As such, in the event of their inadvertent introduction to the Earth's biosphere, it is generally accepted that there would be little threat of widespread ecological disruption because of limiting nutritional or physical constraints or the inability to compete for resources in habitable sites where Earth microorganisms are presumably well adapted. Furthermore, in the event of an accidental release, it is highly improbable that any martian organisms would find an acceptable host or habitat in the vicinity of release. There are large uncertainties associated with these assessments and the risk of potentially harmful effects is not zero, thus it will be prudent to screen for the ability of the returned sample to disrupt microbial ecosystems. Although such tests are not routinely done, it would be advisable to design and conduct suitable microcosm tests to screen for potential ecosystem effects or disruption in biogeochemical cycles.

Two types of microcosm tests are recommended, the first designed to assay for disruptions of important representative microbial systems upon addition of martian materials, and the second to determine if any detected or undetected martian biological entities can grow or propagate in selected sterilized microcosms of representative terrestrial ecosystems.

Detailed information and discussions of the various proposed microcosm tests are provided in Appendix A.2.

<u>Criteria for Distribution of Martian Samples</u>

The interpretation of biohazard and life detection test results was addressed and when and under what conditions unsterilized martian materials could be allowed outside strict BSL-4 containment [as described in CDC and NIH, 1993] was discussed. Although details remain to be worked out, agreement was reached on an overall approach to sample handling consistent with SSB recommendations for returned Mars samples [Space Studies Board 1997].

There should be no distribution of any unsterilized materials outside of containment unless and until rigorous analyses has determined that the materials do not contain any biological hazards. Decisions about distribution of sample material should be based on interpretation of the results of both life detection and biohazard tests and focus on the possible presence of a replicating life form, either extant or dormant, as well as indications of biological activity. It is assumed that sample material will never actually be fully released from containment, but rather distributed in an appropriately controlled manner beyond the initial containment facility. Such controlled distribution will only take place after a thorough review of all life detection and biohazard findings by an appropriate scientific advisory panel. If any portion of the sample is to be removed from containment prior to completion of these analyses, it should first be sterilized, as recommended by the SSB report [Space Studies Board 1997]. When the scientific advisory panel can demonstrate that the martian samples do not represent a biohazard, subsequent conditions for the safe transfer and handling of materials will be dictated by scientific objectives rather than biosafety concerns. In the event that obvious fossil life forms (non-replicating) are found in the sample, consideration should be given to the release of sample materials in a manner similar to those for meteorites or other geological samples.

It is instructive to consider the many possible outcomes of life detection and biohazard testing. Table 1 provides an overview of the various combinations that were considered.

If any life forms are detected, even if preliminary tests suggest they do not pose a biohazard, continued strict containment, rather than controlled distribution, is advised initially. There was consensus that strict containment should be maintained in light of positive test results until the findings are verified by subsequent testing and/or a scientific panel is convened to make decisions about subsequent sample handling. In the event of positive findings, verification testing should be undertaken to confirm initial findings. Such testing should use only in vitro tests under BSL-4 containment. Any decisions to allow either in vivo tests or modifications of containment requirements should be made by an appropriate scientific advisory panel after the required second round of verification tests.

No consensus was reached on what containment/release recommendations should be made if all life detection and biohazard test results are negative. Discussions centered mainly around the issue of what level of scientific uncertainty would be acceptable before containment restrictions could be eased. Basically, debate focused on whether samples should subsequently be handled like geological samples if all findings are negative, or whether a more conservative approach of downgrading to BSL-3 containment should be recommended for some finite period of time to allow additional scientific scrutiny. It became obvious that additional discussion would be needed in order to translate the various test outcomes into specific recommendations for release of unsterilized materials from containment.

Research and Technology Needs

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

1. Validation of Methodological Approach: Research is needed to validate the proposed approach to biohazard testing that emphasizes cells and tissues rather than whole organism studies which were utilized during the Apollo biohazard testing. Prior to the return of the martian samples, methods should be pre-tested thoroughly (e.g., test with known samples containing live organisms to demonstrate their effectiveness; replicate multiple times under conditions identical to those that will be used with the martian samples, etc.) Appropriate changes to procedures should be implemented and practiced to insure that there will be a minimum of procedural difficulties when the martian samples are examined. Techniques that will be used to characterize any isolated or suspected life forms must be developed and tested well in advance.

2. Microcosm Research: Research in the disruption of biogeochemical cycles should be undertaken to determine the effectiveness of proposed microcosms. This validation needs to include an establishment of the predictive value of the microcosm, i.e., how well does the selected microcosm predict what occurs in nature. It will also be necessary to design microcosms that will permit non-destructive sampling of the microcosm. This will allow long

Table 1: Preliminary thoughts on containment/release questions.

If Life Detection Tests are:	AND If Biohazard Tests are:	Containment/Release Suggestions	Action
Incomplete	Incomplete	Safely controlled distribution OK, but only if sterilized first (as per SSB).	Research needed on appropriate sterilization methods.
Negative	Negative	No consensus reached. Suggestions ranged from: a) controlled distribution like geological samples, or b) require at least BSL-3 containment as part of controlled distribution.	Needs considerable further discussion.
Negative	Positive	Retain in strict containment. Controlled distribution not recommended.*	Further *in vitro* tests in strict containment and review by Scientific Advisory Panel (SAP).
Positive	Negative	Two scenarios to consider: a) Extant or dormant life detected. Retain in strict containment; controlled distribution not recommended;* b) Obvious fossil life forms detected; controlled distribution may be allowed.	a) Further in vitro test in strict containment and review by SAP; b) review by SAP, verify as fossilization, treat same as negative findings
Positive	Positive	Retain in strict containment.*	Further in vitro test in strict containment. Controlled distribution and/or in vivo tests not recommended.

* Still need to address question of controlled distribution of sterilized sub-samples.

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term (30-90 days) observation of microcosms inoculated with sample material. It might be useful to examine the microbial community present in selected microcosms under the influence of available martian meteorites.

3. Representative Samples, Controls, and Replicates: Identifying what comprises a representative sample of returned martian materials will become especially important with the inclusion of both rock and soil (see Appendix A.3). Recent meteorite results and the discovery of microbes in deep subsurface rocks on Earth indicate the importance to carefully screen rock interiors. As planned, only a small proportion of returned materials will be used for biohazard testing. Thus, decisions about release from containment will require extrapolation to the untested materials. In addition to the question about a representative sample, attention should also be directed to what constitutes suitable controls and replicates in light of the small amount of sample that will be available for preliminary screening. Research based on combined petrological, chemical, and microbial analyses of appropriate terrestrial analogs may provide guidance as sampling protocols are developed.

4. Other Operational Issues Assessment: To avoid operational and management problems previously experienced during the Apollo sample handling, special attention will be given well in advance to plans for training programs, monitoring of lab personnel, management of lab operations and facilities, and other operational aspects of the sample test facility. To minimize the effects of the limited quantities of material, preliminary sample allocation and access needs to be addressed in advance of the recovery of the samples (e.g., amount of sample to be allocated for quarantine tests and scientific studies; access to samples for research; criteria to be applied to evaluate proposals; etc.)

References

Allton, J.H., J. Bagby, and P. Stabekis, "Lessons Learned During Apollo Lunar Sample Quarantine and Sample Curation," *Adv. In Space Res.* (in press, 1997).

CDC and NIH, *Biosafety in Microbiological and Biomedical Laboratories* (3rd. ed.), HHS Publication No. CDC 93-8395. US Government Printing Office, Washington, D.C. (1993).

DeVincenzi, D.L. and J.R. Bagby, *Orbiting Quarantine Facility: The Antaeus Report*, NASA SP-454, Washington D.C. (1981).

Nealson, K.H., "Sediment Bacteria: Who's There, What are they Doing, and What's New?" *Annu. Rev. Earth Planet. Sci.* 25:403-34 (1997).

Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C. (1997).

APPENDICES

A. Proposed Biohazard Test Protocols

The following sections provide some preliminary thoughts and suggestions for design of pathogenicity test protocols (Appendix A.1), for microcosm studies to detect ecosystem effects (Appendix A.2), and for the selection of representative samples from returned soils and rocks (Appendix A.3). Because the specific details of biohazard tests will undoubtedly evolve over time, and because new methods will undoubtedly be developed, all suggestions and quantitative details below should be recognized as preliminary and subject to modification, especially those related to numbers and types of controls and replicates. With only a limited quantity of material that will be available for biohazard testing, much more discussion is needed to determine what constitutes a reasonable test quantity for appropriate levels of sensitivity and meaningful results.

Appendix A.1: Proposed Pathogenicity Testing

1. Microbiological Culture Media:

    a. Objective: To determine if martian sample materials contain agents capable of replication in microbiological culture media.

    b. Procedure: Inoculate a series of microbiological liquid culture media with organic and inorganic energy sources. Incubate under aerobic, microaerophilic (i.e. reduced oxygen content) and anaerobic conditions, including a CO_2 atmosphere, at temperatures that range from 4 °C to 55 °C. Test to determine that the microbiological liquid culture media can support the growth of molds, photosynthetic bacteria and algae.

    The organic media should cover a range from rich (such as Brain Heart infusion Agar (BHI)) to mineral salts media (with a minimal organic carbon source). The media used to detect chemolithotrophic microbes should include the following inorganic energy substrates: ammonia, nitrates, nitrites, hydrogen, sulfur, sulfide, ferrous ion, thiocyanate, and carbon monoxide. The media should also include the following terminal electron acceptors: carbon dioxide, nitric oxide, nitrous oxide, nitrate, nitrite, sulfate, sulfite, tellurite, selenite, and tetrathionate, and oxygen. Headspace gas analyses should also be conducted on cultures.

2. Cell Culture Inoculation:

    a. Objective: To determine if martian soil contains agent(s) capable of replicating in cell culture.

    b. Procedure: The following types of cell cultures will be used: one fish, one insect, and one monkey cell line and two cell lines from domestic animals and humans. All preparation of inoculum, cell culture inoculation and passage, and agent characterization will be performed in a BSL-4 laboratory. All cell cultures will be tested for the presence of contaminants. Growth and maintenance media will not contain antibiotics. Suspensions of martian soil will be

prepared in cell culture media and the cell cultures inoculated. An equal number of uninoculated control cultures will be prepared and handled under the same conditions. All cultures will be observed daily for cytopathic effect (CPE). At the end of 10 days, cells will be removed and a second passage performed. If no cytopathic effect is observed at the end of 10 days, the study will be terminated. If cytopathic effect is observed in the cell culture, the agent will be characterized using appropriate described techniques (to be developed) and additional passages in cell cultures will also be made. Concurrently, cell culture media will be examined microscopically for extracellular presence and multiplication of prokaryotic/eukaryotic microbial entities.

3. Embryonating Chicken Egg Inoculation:

a. Objective: To determine if martian soil contains agents capable of infecting embryonic chicken eggs, which are susceptible to most bacteria, viruses and fungi.

b. Procedure: Work will be done under BSL-4 conditions. A suspension of martian soil will be prepared in Tris buffered tryptose broth or other suitable media without antibiotics. Eighteen specific-pathogen-free 6-day embryos will be inoculated with 0.2 mL of the suspension by the yolk sac route. An equal number of control embryonating chicken eggs will be inoculated with media only. All embryos will be observed daily. All embryos that die and all the live embryos at 10 days post inoculation will be harvested, a suspension prepared and a second passage performed. All dead embryos on the second passage will be passed again. If embryo death pattern appears to be due to infection with an agent, appropriate identification procedures will be utilized.

4. Mouse Inoculation:

a. Objective: To determine if martian soil contains an agent capable of producing disease or death in specific-pathogen-free mice. (Note: This procedure will only be done if and when all life detection and biohazard tests have been completed and found to be negative. In such case, this inoculation may be carried out under BSL-3 conditions.)

b. Procedure: A suspension of martian soil will be prepared in appropriate media without antibiotics. Twelve specific-pathogen-free mice and twelve control mice will be inoculated with media. All mice will be observed for evidence of infection. If any mice die, a post mortem will be performed and a suspension with brain, liver, lung and spleen will be made and transferred into 6 additional mice. If additional mice die, suitable agent identification procedures will be performed. All agent identification procedures will be performed under BSL-4 conditions. If no mice die due to agents in the martian soil, the test is completed.

5. Test Protists:

Tetrahymena and other representative protists should be screened for the possible pathogenicity of martian materials. The rationale for selecting these test organisms is based on their biochemical similarity with metazoans and humans. Various screening techniques

should be used following exposure/inoculation of organisms to sample materials (e.g., microscopy, cell abundance, effects on growth, etc.) Details for these tests to be developed.

6. Plant Tissue Culture:

a. Objective: To test for the presence of potential plant pathogens in martian sample materials.

b. Procedures: It is recommended that an initial screen be done with both suspension and callus cultures of tobacco, potato and rice. Aqueous suspensions of martian soil will be introduced into the callus cultures which are maintained under light and temperature conditions conducive to growth of the plant.

Regular observations can then be made on the general health of the plant tissues. Additional measurements should include changes in respiration, cell growth (i.e., quantitative and qualitative) and metabolism of a given radiolabeled carbon source.

Appendix A.2: Proposed Use of Microcosms to Detect Potential Ecosystem Effects and Disruptions of Biogeochemical Cycles

To examine the potential ecological implications of martian life to Earth habitats, properly designed and calibrated microcosms can be used effectively to examine the potential effect of exogenous microorganisms on indigenous Earth biota.

Several types of microcosms are possible. The most general approach to biohazard assessment is to 'seed' small-scale closed biospheres ("microcosms") with the samples to be tested, followed by comparisons with un-seeded controls. Types of biospheres include aquaria and terraria and may be confined to microbes or may contain larger multicellular animals and plants. The gas phase of the biosphere (the atmosphere) is the most sensitive to test by GC-MS analysis. If the mass of unknown seed material is small compared to the biosphere mass, continuous differences between tests and control biospheres must be attributed to biota in the test material which have found a niche in the biosphere. Such exotic biota must then be characterized and subjected to biohazard tests. Anything that can alter an ecosystem must be regarded as a potential biohazard. These tests are described as follows:

1. Soil Cores with Wheat Seeds: It is suggested that some preliminary testing should be done with microcosms that consist of intact soil cores. After the cores have equilibrated, surface sterilized wheat seeds will be planted and "inoculated" with a small sample of martian soil. Controls will consist of both intact soil cores with non-inoculated wheat seeds and cores with seeds inoculated with sterilized martian soil.

Some of the parameters to be examined include what potential effect the introduced soil will have on the indigenous microbial community. Approaches may include direct extraction and analysis of fatty acids or nucleic acids to obtain a signature of the microbial community in the treated and control microcosms. It also would be informative to enumerate populations of several groups of microorganisms including gram negative and gram positive bacteria,

actinomycetes, and fungal populations. This portion of the examination also may include the use of both selective and semi-selective media to enumerate specific genera or even species. These measurements need to be made on both bulk soil and rhizosphere populations.

2. Microbial microcosms hazard testing:

a. Martian material will be added to selected microbial ecosystems in small terraria or aquaria containing selected terrestrial soil, mud, sludge, etc. which can test for:

- Disruption of anaerobic electron acceptors (e.g. nitrate, Fe III, sulfate, methanogenesis),
- Disruption of chemoautotrophic microbial microcosms (Fe [II] oxide, sulfide oxidation, Mn^{2+} oxide, methane oxide, nitrification),
- Disruption of photosynthetic microcosms that include microbial mats, photosynthetic bacteria, cyanobacteria, etc., and
- Disruption of diazotrophic (nitrogen fixation) systems.

These systems can be assayed with a battery of simple tests (gas chromatography, radiotracer techniques, pigment/biomass measurements). Controls will include unamended samples and samples amended with sterilized martian material.

b. Hazard tests for growth of martian material should also be conducted in sterilized terrestrial ecosystems, e.g., sterilize the microbial microcosms and inoculate with martian material. This will determine whether martian microbes can grow in terrestrial microbial ecosystems and therefore may represent a propagation hazard.

Appendix A.3: Representative Samples for Planetary Protection Protocol

The initial set of experiments designed to evaluate the potential biological hazard of the returned Mars sample would likely be performed on roughly 10% of the sample, for the following reasons:

1. Only a limited mass of rock cores will be returned by the mission (approx. 500 gm) and pristine material will be required for other scientific investigations and for archival purposes.

2. The biomass present in the rock will be low, based upon organic carbon analyses of martian meteorites. It will also be heterogeneously distributed, based upon observations of microbial distributions in oligotrophic terrestrial environments. At least 10% of the material will be required for testing.

To be certain that the results of the biohazard experiments are applicable to the remaining 90% of the Mars sample, the samples selected for biohazard evaluation should be "representative" of the different types of rock environments. Characterization of the rock environments preserved in the core samples will proceed as follows:

1. Removal of cores from rover core catcher or return canister.

2. Photographic documentation (digital camera), sample mass, magnetic susceptibility, radioactivity?

3. Petrologic documentation by binocular microscope/CCD camera.

4. Preservation at liquid N_2 temperatures until the sample is processed.

Based upon this visual classification, segments of the core will be selected as "representative" of the variety of rock environments present. Some of the factors germane to this categorization are:

1. Location along core length, where core bottom will provide the greatest shielding from UV while core near-surface may harbor sites for endolithic communities (depending upon rock transmissivity-particularly in the visible and near-IR).

2. Core top surface, which presumably provides the maximum UV exposure and is possibly a sterile environment.

3. Core top color or mineral staining, which might indicate biological or abiotic processes.

4. Alteration of the rock matrix, which may indicate the presence of liquid water during the sample's geological history.

5. Mineralized fractures, which could provide porosity and enhanced nutrient transport.

6. Cavities or open pores, which could indicated trapped gas (e.g. vesicles) or secondary porosity developed by dissolution in a liquid water regime.

7. Presence of reduced phases (i.e. sulfide, magnetite or dark particulate material), which could indicate potential respiration products or electron donors.

8. Presence of oxidized phases.

9. Presence of layering and potential chemical and/or redox gradients.

10. Grain size and porosity distribution estimates.

11. Mineral matrix composition and volume.

Once a selection of representative samples is made, priority should be given to those rock or soil environments most likely to harbor life.

The samples for testing will be removed by mechanical splitting (if they are intact cores) and the tracer concentrations (if tracers are utilized) evaluated. The tracer concentrations will be used to evaluate the potential for sample contamination by terrestrial microbes and to classify the samples for subsequent biohazard evaluation.

B. Workshop Agenda

Day 1: Wednesday June 4

9:00 am	Introduction and Welcome - Donald DeVincenzi
9:20	Physical and Chemical Properties of Mars - Ben Clark
10:00	Chemical and Biological Studies of Mars Meteorites - Carl Allen
10:40	Break
11:00	Mars Sample Return Mission Design - Mark Adler
11:40	SSB Guidelines for Prevention of Back Contamination - Margaret Race
12:20 pm	Lunch
1:40	Apollo Experiences: Quarantine, Bioassays, Hazard Testing - Judy Allton
2:20	Modern Techniques for Containment of Pathogens - Peter Jahrling
3:00	Break
3:20	Identification of Biological Entities in Unknown Samples - Alvin Fox
4:00	Modern Techniques for Testing Pathogenicity and Hazards - Joseph McDade
4:40	Subgroup Instructions/Assignments for Day 2 - Donald DeVincenzi
5:00	Group Adjourns

Day 2: Thursday, June 5

8:30 am	Reconvene - Instructions for Subgroups etc.
8:40	Divide into 3 Subgroups
12:30 pm	Lunch
1:45	Subgroups Reconvene
3:15	Break
3:30	Subgroups Reconvene
5:00	Subgroups Adjourn

Day 3: Friday June 6

8:30 am	Reconvene as one large group
	Summary Presentations, Discussion, Recommendations etc.
10:15	Break
12:00 noon	Workshop Adjourns

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13. ABSTRACT *(Maximum 200 words)*

In 1996, several NASA-sponsored studies were underway to look at various aspects of a Mars Sample Return (MSR) mission. One of these studies, performed by the Mars Exploration Long Term Science Working Group (MELTSWG), looked at many issues for MSR including Planetary Protection (PP), both forward and back contamination aspects. One outcome of the study was the realization that little detailed information existed in certain PP-related areas that could be used by mission planners to more accurately design and cost MSR mission concepts. Therefore, the MELTSWG group recommended that NASA fund an effort to look at these PP issues in more detail. A joint Ames Research Center-Jet Propulsion Laboratory-Johnson Space Center proposal was prepared, submitted to NASA Headquarters, and funded. It contained 5 tasks, each of which dealt with a specific PP element for a MSR mission: 1) definition of the environmental impact review process; 2) determination of outbound PP requirements; 3) examination of sample containment technology; 4) development of concepts for ensuring that uncontained Mars material would not be brought to Earth; and 5) development of guidelines for returned sample containment and quarantine analysis. The Workshop on Mars Sample Quarantine Protocol was conducted to address the fifth objective; it was convened at NASA Ames Research Center, June 4-6, 1997.

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