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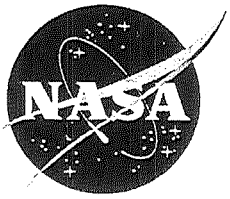

Mars Sample Handling Protocol Workshop Series

Workshop 4 Final Report and Proceedings
Arlington, Virginia
June 5-7, 2001

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June 2002

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PREFACE

Numerous NASA reports and studies have identified Planetary Protection (PP) as an important part of a Mars Sample Return mission, both for preventing forward- and back-contamination and for ensuring maximal return of scientific information. A key element of PP for sample return missions is the development of guidelines for returned sample containment, 'biomarker' analysis, and biohazard testing. Reports from two previous major studies *[DeVincenzi et al., 1999, and Carr et al., 1999]* have provided preliminary recommendations on specific aspects of handling returned Mars samples including biocontainment, life detection, biohazard testing, sample collection and transportation, certification, and sample receiving, curation, and distribution.

To further refine the requirements for sample hazard testing and the criteria for subsequent release of sample materials from containment, the NASA Planetary Protection Officer convened an additional Workshop Series beginning in March 2000. The overall goal of the Series was to develop a comprehensive protocol to assess the returned materials for any biological hazard(s) and to safeguard the purity of the samples from possible terrestrial contamination. It is anticipated that the findings of this workshop series will: 1) assist NASA's Planetary Protection Officer and senior administrators in preparing for Mars sample return facilities, technology, and operations; 2) serve as a briefing document for advisory groups, regulatory agencies, and other entities that will ultimately establish and review sample return handling policies, requirements, and implementation, and 3) provide recommendations in a form suitable as input for possible future announcements of opportunity soliciting proposals for Mars sample handling.

This document is the report of Workshop 4, the last Workshop in this Series; it builds on the deliberations and findings of the earlier Workshops in the Series[1] (Workshop 1: *Race and Rummel, 2000;* Workshop 2*: Race et al., 2001a;* Workshop 2a: *Bruch et al., 2001;* Workshop 3: *Race et al., 2001b*). The reports of these Workshops are available from the National Technical Information Service as indicated on the previous page.

Because development of the Working Draft Protocol was accomplished through an iterative process of discussion and review during the Workshop series and afterwards, it is useful for the reader be aware of key workshops, reviews, publications, and terminology used to refer to various versions of the developing protocol. The table on the next page shows a chronological listing of the stages and terminology used in the process leading to the Working Draft Protocol found in this document in Appendix A (beginning on page 71). Full citations for the various published reports are listed in Appendix F.

1. The reader is referred to the final reports from the prior Workshops in the Series for full documentation of the detailed discussions held by the Sub-groups in those Workshops. As a framework and proof-of-concept, the Working Draft Protocol is a distillation of those discussions and therefore does not include the level of detail brought out in those discussions.

Terminology Used	Date/Location	Report Citation or Annotation
Workshop 1 Final Report	March 2000, Bethesda, MD	*Race and Rummel, 2000*
Workshop 2 Final Report	October 2000, Bethesda, MD	*Race et al., 2001a*
Workshop 2a Final Report	November 2000, Rosslyn, VA	*Bruch et al., 2001*
Workshop 3 Final Report	March 2001, San Diego, CA	*Race et al., 2001b*
Penultimate Working Draft Protocol	Prepared May 2001	First compilation of the developing protocol from recommendations of Workshops 1, 2, 2a, and 3 (not formally published)
SSB/COMPLEX Report: The Quarantine and Certification of Martian Samples	May 2001 Advance Copy	*SSB 2002*
Workshop 4 Final Report	June 2001, Arlington, VA	This document (NASA–CP–211841)
Completed Working Draft Protocol	Prepared June 2001	A consensus working draft resulting from the entire Workshop Series and submitted to the ORC for comment and review (see Appendix A, beginning on page 71 of this document).
Oversight & Review Committee (ORC) review process Oct-Nov 2001	12 November, 2001, ORC Meeting, Rockefeller University New York, NY	Review of the Completed Working Draft Protocol.
Final Version of the Draft Protocol	October 2002	The final version of the Draft Protocol incorporating comments and recommendations from the ORC *[Rummel et al., 2002 in press].*

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EXECUTIVE SUMMARY

In preparation for missions to Mars that will involve the return of samples to Earth, it will be necessary to prepare for the receiving, handling, testing, distributing, and archiving of martian materials here on Earth. Previous groups and committees have studied selected aspects of sample return activities, but specific detailed protocols for the handling and testing of returned samples must still be developed. To further refine the requirements for sample hazard testing and to develop the criteria for subsequent release of sample materials from containment, NASA's Planetary Protection Officer convened the Mars Sample Handling Protocol (MSHP) Workshop Series in 2000-2001. The overall objective of the Workshop Series was to produce a draft protocol by which returned martian sample materials could be assessed for biological hazards and examined for evidence of life (extant or extinct) while safeguarding the purity of the samples from possible terrestrial contamination.

This report provides the first complete presentation of a Draft Protocol for Mars Sample Handling to meet planetary protection needs (see Appendix A, page 71) and a record of the proceedings of Workshop 4, the final Workshop of the Series, which was held in Arlington, Virginia, June 5-7, 2001.

During Workshop 4, the Sub-groups were provided with a draft of the protocol dated May 2001 which had been compiled from work done at all prior Workshops in the Series.[2] Then eight sub-groups were formed to discuss the following assigned topics:

- Review and Assess the Draft Protocol for Physical/Chemical Testing
- Review and Assess the Draft Protocol for Life Detection Testing
- Review and Assess the Draft Protocol for Biohazard Testing
- Environmental and Health Monitoring and Safety Issues
- Requirements of the Draft Protocol for Facilities and Equipment
- Contingency Planning for Different Outcomes of the Draft Protocol
- Personnel Management Considerations in Implementation of the Draft Protocol
- Draft Protocol Implementation Process and Update Concepts

Summaries of the Sub-group discussions and findings begin on the next page; the complete individual Sub-group reports can be found beginning on page 19.[3]

The "Working Draft Protocol" included in this document in Appendix A is based on the deliberations and recommendations of the Workshop 4 Sub-groups combined with those of the Sub-groups from all the earlier Workshops in the Series. As such, it represents a consensus that emerged from the discussions of all the sub-groups assembled over the course of the five Workshops of the Series. These discussions converged on a conceptual approach to sample handling, as well as on specific

2. Development of the Draft Protocol occurred over the course of the Workshop Series; a table of all the versions is shown in the Preface. Prior to Workshop 4, materials developed in all the earlier Workshops in the Series were compiled into a version of the Draft Protocol subsequently designated the "Penultimate Working Draft Protocol" (dated May 2001). The "Working Draft Protocol" (dated June 2001), found in this report in Appendix A beginning on page 71, is the result of incorporating the comments and changes from the Workshop 4 Sub-groups into the Penultimate Working Draft Protocol.

3. Materials such as the Workshop agenda and participant lists as well as complete citations of all references and a glossary of terms and acronyms appear in the Appendices.

analytical requirements. Discussions also identified important issues requiring further consideration, as well as research and development needed for implementation of the final protocol.

The Working Draft Protocol is divided into 3 major analytical areas: physical/chemical characterization of the sample(s), life detection, and detection of any biohazards, if present. It also addresses environmental, and health monitoring and safety issues, facilities and equipment requirements, personnel management considerations, contingency planning for different outcomes of the analyses, and the process by which the final protocol will be updated and implemented. The Working Draft Protocol deliberately lacks detail about specific analytical techniques to be used for testing returned sample materials. Throughout the Workshop Series the sub-groups agreed that the rate of development of new techniques and refinement of current standard techniques is so rapid that these details are best left unspecified until closer to the time of the implementation of the actual final protocol.

This Working Draft Protocol will now undergo a thorough review and refinement process,[4] the first step of which is a review by the Oversight and Review Committee (see Appendix D3, page 145) scheduled to occur in November 2001.[5] The development of the final, detailed sample handling protocol is still a long way off, nevertheless this *Working Draft Protocol* represents a comprehensive and coherent approach to the handling of returned Mars samples. *If any portion of this Working Draft Protocol is to be cited or referenced, it must be with the understanding that this document is not indicative of any final decisions or plans for future Mars missions and that this is not the final version of the Draft Protocol.*

Finally, during a one-hour plenary session on the final day of Workshop 4, the participants explored the areas of research and development needed in order to implement or enhance the implementation of the protocol; these are outlined beginning on page 68. These areas currently exist in various degrees of development and no attempt was made to define specifications or requirements at this time.

Sub-Group 1: Review and Assess the Draft Protocol for Physical/Chemical Testing

Sub-group 1 reviewed, assessed, and adjusted the Penultimate Working Draft Protocol for Physical Chemical (P/C) processing, which was compiled from deliberations and recommendations in earlier Workshops in the Series. The proposed processing plan is based on a solid heritage of P/C handling, processing, and experiences with Apollo lunar samples, Antarctic meteorites, and cosmic dust. The processing plan is consistent with requirements and conditions recommended by the Space Studies Board (SSB) of the National Academy of Sciences *[SSB 1997],* the Mars Sample Handling and Requirements Panel (MSHARP) *[Carr et al., 1999],* and the Curation and Analysis Planning Team for Extraterrestrial Materials (CAPTEM) *[Neal, 2000].* As a starting position, the Sub-group determined it was essential to examine all the returned material in at least a minimal fashion in order to provide enough data to make informed choices about samples for subsequent Life Detection (LD) and

4. The reader should understand that this Working Draft Protocol represents an early stage in the development of the final protocol, and is reported here to document this stage in the process.

5. At the time this report went to press, the ORC had met; the Final Version of the Draft Protocol *[Rummel et al., 2002, in press],* with their comments incorporated is expected to be published in October 2002.

Biohazard (BH) testing. In recognition of the rate of technological and scientific change anticipated in both LD and BH areas, the proposed P/C plan provides a conceptual approach to processing and handling rather than a detailed list of prescribed methodologies based on current approaches. The Sub-group recommended the use of a Scientific Oversight Committee at the time of sample return in order to adjust the P/C processes to changing technology and mission specifics, to monitor the final protocol in progress, and to make real-time adjustments to fit the actual returned materials.

Overall, the P/C processing can be divided into three phases: 1) initial pre-processing prior to preliminary examination of samples; 2) preliminary examination and screening to permit informed choices for selecting samples for subsequent detailed testing; and 3) subdivision of selected samples for LD and BH tests. The actual P/C processing is made up of three main tracks – one each for gases, fines, and solids. In addition, there are provisions for creating carefully controlled storage and reserve banks for both pristine and handled sample materials.

The P/C process begins with pre-processing steps to clean, decontaminate, and open the sample return canister (SRC), followed by prompt extraction of head gas and back-filling with a chemically un-reactive gas to ambient 'room' pressure. Head gas and other gas samples will be distributed in sealed containers after suitable filtration to remove any particles (filter size TBD).

Processing of solids (regolith samples, rocks, rock cores, soil cores, etc.) includes the requirement for careful consideration of appropriate ambient conditions and materials to which the samples will be exposed. After preliminary examination, rock fragments and cores will be separated from fines and sorted into groups for preliminary characterization and screening, and eventual selection of materials for LD and BH tests. Samples and sub-samples not selected for further screening at this point will be stored in a pristine sample bank designed to maintain the physical, chemical, and biological integrity of samples under controlled conditions while they await allocation for other analyses and research at a later date.

The fines track is designed to process samples of varied types (collected dusts, regolith with coarse materials removed, dusts filtered from head gas, or particulates removed from surfaces of rocks or cores.) After characterization by imagery, fines will be split and selected for LD and BH assays.

Real-time modifications to the P/C process are likely, and will be done in close collaboration and consultation with those involved in LD and BH processing. The Sub-group report also includes a detailed list of future research and development needs and specific areas of concern.

Sub-Group 2: Review and Assess the Draft Protocol for Life Detection Testing

Sub-group 2 examined those sections of the Penultimate Working Draft Protocol related to Life Detection, and suggested modifications to wording and content in several sections that had been developed earlier.[6] In particular, refinements and revisions were made in sections related to the *Principles* guiding Life Detection tests, *Analytical Methods* to be used, and *Integration and Organization of Methods*. In addition, the Sub-group included commentary and revisions on other

6. The full report of Sub-group 2 (beginning on page 33) was prepared in the form of comments and suggested changes to the Penultimate Working Draft Protocol. No attempt was made to re-write their report as prose.

areas of concern including prioritization of analytical methods, controls, equivocal findings, sensitivity, detection of biological activity, culture testing, non-carbon based life, and possible independent origins of martian life.

In the text related to *Principles Guiding Life Detection* tests, the Sub-group suggested refinements to emphasize broadly-defined signatures (e.g., structural, chemical-structural and biosynthetic, isotopic, and geochemical) that cover all known terrestrial life as well as, possibly, non-terrestrial life. In addition, revisions were made to earlier versions of tables to clarify wording about pre-biotic chemistry, universal properties of life, and non-Earth-based life.

The Sub-group made modifications to the *Analytical Methods* to be used and recommended a general approach that initially surveys a portion of different sample types for suggestive features – structure, basic chemistry (organic or complex carbon), and local inhomogeneites – followed by focussed examination for polymers and more complex structures. Clarifications and refinements were also made about sample survey methods, culturing efforts, and the search for and interpretation of geochemical signatures of life and discrete subsets of compounds.

Sub-group 2 also suggested modifications concerning *Integration and Organization of Methods* within the Life Detection section. Specific changes were made on the use of minimally destructive methods, a recommendation to start testing with surface fines, and the need for more details about controls, transport procedures, forward contamination, and false positives.

The Sub-group added comments concerning the *Use of Replicate Analyses* to seek bioactivity through time, and addressed problems about sterilization and false positives. The Sub-group also suggested that it is inadvisable at this time to specify a time period for completion of LD testing.

The Sub-group made extensive comments on several other areas as well. Specifically, they provided detailed comments regarding prioritization of analytical methods for Life Detection. The recommended sequence of tests for "signs of life" should be designed to proceed sequentially through analyses for structural, chemical, biochemical and molecular evidence followed by methods searching for replication or signs of bioactivity. Minimally destructive methods to detect structural signs of life should guide the use of methods to seek chemical, biochemical, and molecular signs of life. Sub-group 2 also included comments related to controls, integration of the protocol, sensitivity of methods, microbial culture strategies, and the relative value of specific methods for detecting activity versus inactivity. Finally, Sub-group 2 added comments concerning concepts on the origins of life on Mars and/or Earth and suggested alternative ways of thinking about Life Detection based on biochemical patterns and unusual macromolecular assemblages and compounds.

Sub-Group 3: Review and Assess the Draft Protocol for Biohazard Testing

Sub-group 3 examined those sections of the Penultimate Working Draft Protocol related to Biohazard (BH) testing. The format, wording, content, and current limitations were addressed. Since many key issues had already been addressed during previous Workshops, the Sub-group focused on perfecting parts that had already been substantially worked through, and on identifying and addressing pending issues. The specific modifications identified in the Sub-group report have been incorporated in the BH portion of the Working Draft Protocol beginning on page 103.

Sub-group 3 began by addressing the issue of definitions and terminology used in sections on Biohazard testing. For example, changes were made to broaden the definition of biohazard to include both a 'replicating agent' and/or 'one able to be amplified by a biological system.' In addition, wording was changed to include any type of 'significant alteration,' rather than just adverse effects. Terminology for "biohazard" was refined in a way that clarified the meanings and intentions.

The Sub-group suggested a format change in the PWDP for references to the report[7] of the National Academy of Sciences' Committee on Planetary and Lunar Exploration (COMPLEX) *[SSB 2002]* and made suggestions about criteria to be used for selecting tests ten years hence.

For the sake of clarification, and in order to keep a coherent set of definitions throughout the documents (and all further documents), Sub-group 3 also made recommendations related to levels of containment and numbers of facilities. They specifically emphasized the need to use the newly defined planetary protection level (PPL) terminology[8] rather than biosafety level (BSL) and to explicitly define required levels of cleanliness for each level of PPL. In addition, for clarity, the Sub-group recommended the use of "BSL-3-Ag"[9] instead of "BSL-3," with BSL-3-Ag designated as "PPL-δ," to be considered as the first possible downgrading of containment in the event that the results of all LD and BH tests are negative. The Sub-group indicated that only the Sample Receiving Facility (SRF) is likely to require PPL-α, the strictest level of cleanliness and containment coupled with Mars-like ambient conditions. If facilities beyond the SRF are used as part of the final protocol testing, these other facilities will likely be certified for conducting studies and tests at PPL-β, PPL-γ, and PPL-δ conditions. The Sub-group suggested that BSL-3-Ag facilities should be built around large instruments, rather than miniaturizing instruments to fit into a pre-existing lab. Finally, the Sub-group identified the need to certify mobile containers at the appropriate PPL (as opposed to BSL requirements exclusively) to allow transport of samples.

Two divergent points of view emerged during the discussion about possible irradiation of samples, although it was not part of Biohazard testing *per se*. Some Sub-group members argued that even in the absence of evidence of biohazard, samples should be gamma-irradiated at substantial doses (equivalent or higher to those used for present-day BSL-4 sample release) prior to gradual de-containment and release. Those with a different perspective argued that, if Life Detection and Biohazard results allow the conclusion that samples are safe enough for release, there is nothing in them that can be made safer by irradiation. Despite lengthy discussions, Sub-group 3 did not reach any consensus and could not make any recommendations regarding irradiation or sterilization of samples.

Finally, the Sub-group also made several recommendations related to sub-sampling, and noted the importance having explicit decision making procedures for data interpretation and decision making as well as a communication plan to keep both the public and the scientific community informed of results during Life Detection and Biohazard testing.

7. An Advance Copy of the COMPLEX Report was made available to the Workshop 4 participants.
8. For complete definitions of the four PPL levels, see page 74.
9. For the most current definition of BSL-3-Ag, consult the web site of the U.S. Department of Agriculture <http://www.afm.ars.usda.gov/ppweb/242-01m.htm>

Sub-Group 4: Environmental and Health Monitoring and Safety Issues

The charter for Sub-group 4 was to determine methods relevant to the protocol for monitoring the health and safety of the personnel working in the Sample Receiving Facility (SRF) and of the environment in and around the SRF, (as well as at secondary sites, if any are used). The Sub-group considered monitoring of both personnel and environment over time, beginning prior to the arrival of Mars samples, during and after work on the Mars samples at the SRF, and at secondary sites. Also discussed was the question of how long to continue monitoring.

The leading principle for personnel monitoring and safety should be the optimal protection from the anticipated hazards for the individuals working with the Mars samples. Because of the unique nature of the potential hazards, additional controls beyond those routinely used for hazard monitoring may be required. Nevertheless, the monitoring plan should use existing regulations and standards whenever possible, and maintain a balance between the estimated risks to individuals, the environment or the general population, and the personal impositions of the monitoring program. The plan should allow for cross-correlation of data from the LD and BH testing with data from monitoring of the SRF personnel and environment. Procedures to monitor personnel and the environment should be developed considering international regulatory, cultural and ethical issues. These procedures for the monitoring of personnel should include education and certification.

Five categories of potential hazards were considered in discussions: physical hazards (radiation from samples and hazards associated with equipment); potential chemical hazards from non-biological toxins; biological hazards; psychological hazards (working under PPL conditions); and monitoring of containment itself. Recommendations for monitoring are as follows:

Physical Hazard Monitoring: Physical hazard monitoring should be among the first P/C tests done. If the radioactivity level does not represent a biohazard, then radiation monitoring can be discontinued, unless required for equipment used in the SRF. Other risks from equipment or facilities can be addressed by standard procedures of training and maintenance.

Chemical Hazard Monitoring: Chemical hazard monitoring will be assessed early in the P/C testing process. If an unusual substance or chemical is identified, specific monitoring methods can be designed, and the substance might also be used as a marker for breach of containment monitoring.

Monitoring of Containment and the Environment: Standard methods for monitoring containment can be adapted for use in PPL. Procedures can be developed to assess breaches both inside and outside the SRF and to correct personnel exposures as well as possible environmental and/or human consequences.

Before arrival of a Mars sample, a assessment of the environment around the SRF should be made, identifying sentinel species (i.e., microbes, insects, plants, animals) for use in monitoring for environmental changes. During sample handling at the SRF, environmental monitoring could focus on the identified sentinel species and any novel components of the Mars samples, if identified. It may also be useful to track and record the daily weather conditions in the area of the SRF as baseline data in case of a breach to the outside or the reporting of any unusual events. After completion of LD and BH testing, the level of continued environmental monitoring should be reassessed.

Monitoring of Personnel: Personnel monitoring should focus on time periods before, during, and after Mars sample testing at the SRF, as well as parallel monitoring at any secondary sites that may be used.

A process of certification for people hired to work in the SRF should be developed that is completed before sample arrival and includes education about procedures and risks for employment, security clearance, and medical examinations and tests. Clear inclusion and exclusion criteria should be developed prior to the hiring of personnel. Baseline medical evaluations should use existing standards appropriate at the time the evaluations are performed, with recommended baseline evaluations to include a medical history, physical examination, tests on the person (e.g., chest X-ray), tests on samples from the person (e.g., blood and urine), and neuro-psychological evaluations. All testing should be as non-invasive as possible and maintain a balance between estimated risks from the Mars samples and the risks associated with the tests.

A schedule for regular evaluations of personnel during sample handling should be established, using the same evaluation methods as in baseline data collection. Procedures for medical management of personnel illnesses should be available either on site or with adequate transportation to a medical facility (with BSL-4 containment if needed). Guidelines should be developed for the various scenarios (exposed/symptomatic, exposed/asymptomatic, symptomatic/questionable exposure, etc.) with intervention, treatment plans, containment, and monitoring as appropriate.

The question of how long to continue monitoring after completion of the final protocol was not totally resolved, but will certainly be influenced by the outcomes of LD and BH testing. The issue was raised as to when monitoring moves from risk management to a research study. The suggested level of long term monitoring ranged from minimal (e.g., tracking events like the date and cause of death), to more extensive monitoring (e.g., regular examinations and testing similar to baseline testing). Another question Sub-group 4 raised with little consensus was determining the appropriate control group for comparisons with personnel evaluations.

Monitoring at Secondary Sites: Secondary sites beyond the SRF should be identified prior to the arrival of the Mars samples to allow for pre-certification of personnel and collection of baseline data. All distributions of Mars sample materials must be tracked and procedures for monitoring of containment at the secondary sites should be developed. Personnel at secondary sites should be monitored using the same processes as used at the SRF. The level of monitoring at secondary sites should be based on the results of the Life Detection and Biohazard testing.

Database Issues: A central database facility with data analysis capabilities and procedures should be used to gather and maintain all environmental data (e.g., baseline, monitoring), personnel data (e.g., baseline, in-process, follow-up), secondary site data, and sample tracking data. Processes for regular data analysis, reporting, back-ups, access, and confidentiality should be developed.

Sub-Group 5: Requirements of the Draft Protocol for Facilities and Equipment

Sub-group 5 discussed a series of questions related to the requirements of the protocol for facilities, equipment, and secondary facilities, including the advantages/disadvantages of multiple facilities.

Size and Scope of the Facility: The size and scope of the facility will depend on the decision whether to conduct all protocol tests at the primary SRF site or to distribute protocol functions and activities to secondary labs outside the SRF. The Sub-group reviewed the Penultimate Working Draft Protocol (PWDP) to develop a schematic showing the sequence in which the samples will be processed prior to distribution to the broader scientific community (Figure SG5-1, page 51). Using the PWDP, the Sub-group compiled a rough inventory of the proposed tests and bench top footprint of the requisite instruments. Based on this inventory, the Sub-group suggested that any individual PPL laboratory will not need to be larger than a medium-sized room.[10] Regardless, the SRF must be flexible and able to be expanded.

Where Should the SRF be Located?: The decision of where to locate the SRF was not considered to be a scientific issue; obvious cautions to avoid placement at sites subject to severe natural hazards (e.g., active faults, flood plains, etc.) will be exercised. The Sub-group reviewed the recent National Research Council report on a Mars Facility *[SSB 2002]* and did not concur with their decision that this facility should necessarily be co-located with a BSL-4 facility. The Sub-group favored a location with ready access to an existing labor pool of scientists.

When Should Design of the SRF Be Started?: Regarding the question of when to begin design of the SRF, the Sub-group recommended that the process should be started as soon as the mission is certain and the funds have been allocated. Ideally, given the amount of time required for the process, the design of the SRF should begin at least 10 years prior to the expected sample return.

Distribution of Protocol Activities: In discussing the question of whether or not to distribute activities and functions of the final protocol to more than one laboratory or facility, the Sub-group compiled a list of advantages and disadvantages but made no recommendations. The majority opinion was to limit activities and functions of the final protocol to one major facility, perhaps with the exception of having a duplicate back-up holding facility for the banked Mars samples. However, the Sub-group agreed that there is nothing inherent in planetary protection requirements that would preclude the use of multiple facilities to receive and process these samples. There was also a broad-based consensus on two additional issues: 1) given the anticipated cost and unique design of the SRF, it would be advisable to build the facility for continuous operation to support other astrobiology research activities or those in biological or micro-circuitry sciences; and 2) the SRF must be flexible and able to be expanded.

If a Life-Form/Biohazard is Detected: In the event a life-form/biohazard is detected, the SRF must have the flexibility to quickly expand to accommodate scientific research by principal investigators and researchers. In addition, if a life-form/biohazard is detected, samples should not be released for distribution to the broader scientific community.

10. Details on the sizes and/or number of various PPL labs are yet TBD.

Requirements to be Met by Secondary Facilities: Any secondary facilities must meet the same standard operating procedures as the SRF, (e.g., staff monitoring, security, chain of custody, etc.), and have appropriate PPL containment based on testing needs.

Additional Issues: Sub-group 5 identified a few additional issues and the following recommendations:

- Completely define the PPL containment guidelines.
- Develop schematics for a self-contained containment structure that could be placed in a BSL-4 laboratory and as a composite could meet PPL-α containment requirements and use remote robotics to handle the specimens.
- Develop a comprehensive list of equipment required for all proposed tests in the protocol.
- Anticipate the need to do some Life Detection tests under simulated martian environmental conditions while maintaining PPL-α/β containment.
- Put agreements in place with any anticipated PPL-δ laboratories prior to receipt of Mars samples.

Sub-Group 6: Contingency Planning for Different Outcomes of the Draft Protocol

Sub-group 6 was charged with anticipating how the scientific community would react under a variety of possible scenarios following the return and testing of martian samples. In addition to considering the suggested scenarios in the original charter, the Sub-group also included the question of how to respond in the face of possible breaches in containment.

Lessons From Workshop 3: The Sub-group agreed with the recommendations made in the plenary discussion of Workshop 3 on 'What [to do] if Life is Detected'[11] and identified a number of points relevant to their charge (i.e., need for an Oversight Committee, a Communication Plan, a plan for real-time review of the final protocol, and reviews of facilities and equipment, security concerns, intellectual property rights, international considerations, etc.).

After considering the specific scenarios and questions in its charter, the Sub-group made the following findings and recommendations:

Breach of Containment: The consensus of the Sub-group was that we know how to handle breaches based on long term experience and emergency plans for handling pathogenic biological material under BSL-3 and BSL-4 containment. Additional information for responding to breaches and containment problems has been gained through experience in handling lunar and extraterrestrial materials. Clearly, an emergency plan will be needed well in advance to develop recommended responses to various breach scenarios. The plan should focus on all aspects of mitigation, cleanup, and recovery from the perspectives of both biosafety and sample integrity.

Organic Carbon: It is extremely likely that carbon will be found in sample materials. The sensitivity of current and future methods will be very high, so that at least some degree of contaminants will likely be detected. The existing knowledge on meteorites and other material collected from space will be useful in providing baseline information to help guide the investigations. Since the Viking results

11. See *Race et al. 2001b, page 41* for a discussion held during Workshop 3 on "What if Life is Detected."

focused on volatile organics, *in situ* measurements of non-volatile organics would be useful for predictions of anticipated sample organic content.

Extant Life or Biomarker Positive: If extant life or evidence of biomarkers is detected in the samples, all work on the samples will continue to be done in containment. Maximum effort should be made to determine if the positive results are originating from terrestrial life (i.e., false positives from contamination) or martian life. Finally, if extant life or evidence of past life is detected, information management becomes an issue for both scientific communication and public dissemination of information.

Non-Terrestrial Life Confirmed: If a portion of a sample is confirmed as positive for non-terrestrial life, subsequent testing and analyses on all sample materials will continue in maximum containment. Experimentation on methods to sterilize samples containing the newly discovered life-form should begin in conjunction with investigations of appropriate culture conditions. Once appropriate sterilization procedures can be validated, detailed plans for distribution of samples can be developed or revised in order to meet the established or revised scientific objectives.

Contradictory/Inconsistent Results: Given the number of techniques, spanning several scientific disciplines, it is very likely that contradictory or inconsistent results will be found. Variations in the sensitivity of methods will exist and confidence in the level of controls will differ. It will be important to stress replication of experiments and duplication of results among multiple sites to add confidence to the results obtained.

Application of Release Criteria: The stated goal of this Workshop Series was to devise a Draft Protocol that could rigorously analyze returned martian sample material(s) to determine that those material(s) are free from biohazards and/or and extraterrestrial life-forms and are therefore safe to be released from containment in their native state for further scientific research. Nonetheless, there was disagreement among the Sub-group members on how to handle a sample found to be devoid of organic material and yielding no evidence of life or biohazards.

Despite the recommendations of COMPLEX *[SSB 2002]*, some members of Sub-group 6 felt that an increased assurance should be given prior to any sample distribution and that some sterilization procedure would be advised. A method of 'prophylactic sterilization' was proposed that would involve gamma radiation and minimal heating. Other members of the Sub-group reaffirmed the position that the purpose and design of the protocol is to test for significant biohazards and thus following the final protocol should be sufficient. It was felt that blind additions to the final protocol would destroy some significant scientific value of the sample while unnecessarily eroding public trust, without adding significantly to the assurance of public safety. Because the Sub-group was unable to reach consensus on the question of 'prophylactic sterilization,' the arguments and counter-arguments are presented in their report (see "Text Box 1" and "Text Box 2" pages 55 and 56). Clearly, the issue of sterilization will require serious additional attention and research well in advance of sample return.

Other Considerations – Containment Facilities and Management: Based again on experience with handling of pathogenic biological materials, multiple locations for facility functions may be beneficial for a number of reasons (e.g., redundancy, increase the security of the samples, etc.). The biohazard,

curation, and security equipment are well known, and selection of methods and equipment should be based on current biomedical and counter-bioterrorism techniques.

A Facility Administrator should be present on site to make day-to-day decisions about facility management, acting under the general guidelines of the final protocol established by a scientific oversight committee. This committee should be available as needed by the Facility Administrator, especially for non-anticipated scenarios. Every effort should be made to coordinate the administration of the facility with relevant government agencies.

Sub-Group 7: Personnel Management Considerations in Implementation of the Draft Protocol

Sub-group 7 addressed personnel management and staffing considerations associated with the design and construction of the facility(-ies) and the final implementation of the protocol. After a thorough discussion about the alternative methods for staffing the facility(-ies), the conclusion was reached that various categories of personnel will be required, depending on the different tasks that need to be implemented. The Sub-group agreed to the following several considerations:

- The personnel should be hired progressively during the development of the project and the facility(-ies). The functions and responsibilities of the Director of the SRF may be carried out by appropriate committees until about five years before the return of samples from Mars.
- The required methods and procedures in the final protocol should be applied to any facility or site handling martian samples during the implementation of the final protocol;
- The international character of the program should be respected throughout the whole process.

In their deliberations, the Sub-group developed an overview of the functions, staffing requirements, and organization that will be needed to design, build, and operate an SRF. A series of organizational charts and timelines from now until receipt of samples describe their recommendations (see figures SG7-1 through SG7-4, beginning on page 59). In developing their recommendations for management and staffing of the SRF, the Sub-group used the following assumptions as their working hypotheses:

- The final protocol must be fully and successfully tested before the handling of the actual martian samples.
- It's estimated that a complete series of Experiment Verification Tests (EVTs) will last approximately 6 months and one complete EVT series must be successfully demonstrated before handling of the actual returned Mars samples. The first EVT must begin no later than 18 months before the Mars samples arrive on Earth in order to allow enough time to adjust and repeat the EVT if necessary
- These EVTs are part of the normal operational testing and are consistent with SSB recommendations *[SSB 1997]* and earlier workshops that the SRF be operational two years before the arrival of Mars samples.
- Based on experiences at other BSL-4 laboratories in the United States and France, no less than one-year is required to properly staff and train the technical and scientific personnel.
- Commissioning of the SRF, which must be performed in parallel with the staffing and training, will last at least 18 months.
- In order to accommodate the staffing, training, and commissioning requirements of the SRF, construction of the facility must be finished three and a half years before the actual operations.

- From past experiences in both France and the United States, construction of the facility itself will also require three and a half years.
- It is estimated that about three years will be needed to develop design specifications and plans for the SRF and obtain necessary authorizations for the facility. To accommodate all the activities necessary to design, build and operate an SRF, the entire process must begin fully ten years in advance of sample return.

The Sub-group identified organizational and staffing needs at three key times: 10 years, 5 years, and 3 years prior to sample return, and provided a series of charts outlining the process. Details about key positions, scientific oversight committees, advisory committees, design committees, staffing needs, and working groups are described in the full Sub-group 7 report (beginning on page 57).

Finally, Sub-group 7 also identified three major issues for further consideration:

1. Since no one has experience in simultaneous operations or activities in BSL-4 and clean room conditions (i.e., PPL environment), seeking advice from experts in the pharmaceutical or the microprocessor industries would be helpful.
2. Details on the optimal staffing mix at the SRF must be considered further to ascertain the optimal mix of civil servants, semi-permanent employees, contractors, and guest scientists needed to staff the facility and implement the final protocol. International access and participation should be considered throughout the process.
3. In order to comply with planetary protection constraints and final protocol requirements, a sustained and adequate budget will be needed throughout the design, building, and implementation phases of this project.

Sub-Group 8: Draft Protocol Implementation Process and Update Concepts

Sub-group 8 addressed questions related to the review, approval, updating, and implementation of the final protocol. Before recommending specific processes and committees, the Sub-group highlighted several key issues because of their importance to the overall protocol: the need for clarity of meaning and consistency of terminology; the use of 'PPL' rather than 'BSL' designations; and the creation of an additional containment category, PPL-δ, which is equivalent to BSL-3-Ag.

The Sub-group developed an overview schematic of the implementation process (see Figure SG8-1, page 63) and made the following specific recommendations:

Final Scientific and Policy Reviews: The ultimate review of the final protocol document must be subjected to the highest degree of scientific scrutiny and evaluation conducted jointly by scientific organizations from both the United States and France. This review should probably occur at the level of the National Research Council in the United States, and its equivalent scientific organization in France. Final decisions about which institutions should be involved in scientific reviews are TBD.

Ethical and Public Reviews: Evaluations of the final protocol should be conducted both internal and external to NASA and CNES and the space research communities in the nations participating in the mission. An ethical review should be conducted and made public early in the process (appropriate French and U.S. agencies to conduct the review are TBD). The final protocol should be announced broadly with requests for comments and input from the scientific community and the public.

Future Modifications to the Protocol: When a final protocol has been adopted and approved by a consensus of appropriate scientific organizations, few changes should be made to its content. Changes should be made as scientific information, methodology and/or technology improve between the time of the approval and the actual physical implementation of the final protocol within the SRF. The Sub-group recommended that changes in the final protocol methodology may be considered if a proposed change would meet the following criteria:

- increases the sensitivity or selectivity of the test,
- reduces the length of time necessary for a test without a reduction in sensitivity or selectivity,
- reduces the complexity of the sample handling process,
- increases the overall safety of the process,
- reduces the chances of contamination to the sample or the environment,
- reduces the cost of the process, or
- represents a new technology or method that has the broad general acceptance of the scientific community.

Advisory Committees and Expert Panels: Changes to scientific methodology and instrumentation are inevitable due to the long development time envisioned for this mission. This necessitates long term, consistent input and advice from the external scientific communities. To facilitate this process, it is recommended that a standing Planetary Protection Advisory Committee (PPAC) be appointed in the U.S. to provide input to NASA's Planetary Protection Officer and that a similar standing committee (Planetary Protection Committee, PPC) be appointed in France as currently tentatively planned.

Sub-group 8 also recommends that standing joint French and U.S. working committees or specialized expert panels should be appointed with appropriate expertise to provide support and advice to the U.S. PPAC and French PPC in technical processes, scientific procedures, and safety/biosafety issues. These panels and committees may function jointly or independently depending on the specific need. The U.S. PPAC and French PPC will receive the annual reports of the three panels, who will also provide annual written reviews to NASA's Planetary Protection Officer and, in France, to the appropriate Minister to whom the French PPC reports.

Communications: Since unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels, a communication plan must be developed as early as possible to minimize the dissemination of misinformation and to provide the highest level of public assurance about the issues addressed by the mission. Communications should clearly inform about the extensive efforts to protect the environment and health and safety through facility designs, procedures, and personnel training. This information on risk management and planetary protection should be balanced with education/outreach about the anticipated benefits of Mars exploration and sample return from the scientific perspective. Details about who will be in charge of this plan and the release of information and results are TBD.

Flow Charts and Timelines: In order to assure the rational utilization of both the facilities and sample materials, development of appropriate flow charts and time lines will be needed to coordinate the complex series of interrelated processes and identify key decision points (e.g., release from containment, downgrading to lower level of containment).

Workshops/Reviews: The need to change schedules and procedures may be anticipated during the time between now and sample return. To provide assurance that rules exist between the involved international partners and the scientific communities, two workshop/reviews should be scheduled prior to sample return to Earth in order to reaffirm details about process, methodology, safety and release criteria. The first review should be conducted at the conclusion of the facilities design phase to determine if the physical structure meets the scientific and safety standards as defined within the specifications. A second similar workshop/review should occur after the samples have been collected on Mars but in advance of their actual return to Earth.

Preparations and Processes for Decision Making about Release of Samples: It will be important to make advanced preparations for decision making in the event that a distinctly martian life-form is found within the returned samples. While it is impossible to develop details of the final protocol at this time, it will be important to have considered how decisions will be made, by whom, and based on what principles, if an extraterrestrial life-form is discovered. A specific committee should be established at least one year in advance of sample return to develop contingency protocols and processes that would be in place if and when a martian life is found and verified. It will also be important to have a review and approval infrastructure for decisions about whether or not to release sample materials from containment after the final protocol is completed. The decision to release samples should involve an Interagency Committee on Back Contamination (ICBC) similar to the one used during the Apollo program, as well as the U.S. PPAC and French PPC reporting to relevant bodies in their respective countries.

The organizational structures, management plans, charters and reporting lines for many of the proposed committees and groups will need to be developed in the coming years. Many questions cannot be resolved until additional details on facility design, operational logistics, mission architecture or anticipated schedules are made available.

Research and Development Needs

Throughout the Workshop Series, research and development needs have been identified in the various sub-group discussions and reports. On the final day of Workshop 4, a plenary discussion was held which focused on areas of research and development that need to be pursued to adequately design the SRF and facilitate the implementation of the final protocol. A list of additional research and development concerns was compiled; categories and topics identified during the plenary discussion included:

- Improve controls on samples, tests, personnel, and monitoring
- Refine and improve equipment for using synchrotron tomography
- Develop ecological microcosms
- Study post-radiation detection of biosignatures
- Discussion/descriptions of endolithic community
- Research and characterization of microbiological community cultures
- Robotics and remote manipulation in PPL conditions
- Develop transport and remote containment methodology/requirements
- Improve methods for detection of organic compounds on surfaces

- Miniaturization of testing and assaying techniques as part of the protocol
- Social science research (i.e., personal and community risk psychology)
- Sample register and tracking methodologies
- Communications
- Sterilization of surface adhering bugs
- Remote sharing of data; telepresence
- Research on rock materials using BH testing procedures
- Cognitive 'protheses' – nanobots in diagnostics
- Combined BSL and cleanroom (i.e., PPL) capability
- Appropriate protective gear for staff working in PPL environment.

INTRODUCTION

In preparation for missions to Mars that will involve the return of samples to Earth, it is necessary to prepare for receiving, handling, testing, distributing, and archiving of martian materials here on Earth. Previous groups and committees have studied selected aspects of sample return activities, but specific detailed protocols for handling and testing of returned samples from Mars must still be developed.

To refine the requirements for Mars sample hazard testing and to develop criteria for the subsequent release of sample materials from containment, NASA's Planetary Protection Officer convened the Mars Sample Handling Protocol (MSHP) Workshop Series in 2000-2001. The overall objective of the Workshop Series was to produce a Draft Protocol by which returned martian sample materials could be assessed for biological hazards and examined for evidence of life (extant or extinct), while safeguarding the samples from possible terrestrial contamination.

This document provides the first complete presentation of the Draft Protocol for Mars Sample Handling to meet planetary protection needs (beginning on page 71). Because this version of the Draft Protocol is subject to review and modification by an Oversight and Review Committee (established as part of the protocol-development process), it has been designated the "Working Draft Protocol."[12] The Working Draft Protocol represents a consensus that emerged from the discussions of all the sub-groups assembled over the course of the MSHP Workshop Series which converged on a conceptual approach to sample handling, as well as on specific analytical requirements. Discussions during Workshop 4 also identified important issues remaining to be addressed, including areas where future research and development are necessary for optimal protocol implementation.

This document also provides a complete record of the proceedings and findings of Workshop 4, the final Workshop in the Series,[13] convened June 5-7, 2001 in Arlington, Virginia.[14] The main work of Workshop 4 occurred in sub-group discussions. Workshop participants were provided with a draft of the protocol that had been compiled in May 2001 from work done in all the earlier Workshops in the Series.[15] Workshop 4 participants were divided into sub-groups to address eight separate topics and develop recommendations as appropriate.[16] On Day 1, the Sub-groups' assigned topics were:

- Review and assess the Draft Protocol for Physical/Chemical testing
- Review and assess the Draft Protocol for Life Detection testing
- Review and assess the Draft Protocol for Biohazard testing
- Environmental and Health Monitoring and Safety Issues

12. See the table in the Preface for a complete list of the various versions of the Draft Protocol.
13. Because there was both a Workshop 2 and a Workshop 2a, Workshop 4 is the fifth Workshop in the MSHP Series.
14. The Appendices at the end of this report comprise the agenda, lists of participants, background tutorials presented (in the form of the viewgraphs used by the speakers), complete citations of all references and a glossary of terms and acronyms.
15. Prior to Workshop 4, materials developed in all the earlier Workshops in the Series were compiled into a version of the Draft Protocol subsequently designated the "Penultimate Working Draft Protocol" (dated May 2001). The "Working Draft Protocol" (dated June 2001), found in Appendix A, is the result of incorporating the comments and changes from the Workshop 4 Sub-groups into the Penultimate Working Draft Protocol.
16. The specific charters of each sub-group and their complete summary reports are present in detail beginning on page 19 of this report. The summary reports presented in this document (including tables and figures) reflect the complete deliberations of each sub-group. The views expressed and any conclusions and recommendations reached by the sub-groups do not represent a consensus of all Workshop participants.

After summary reports for each sub-group were presented in a plenary session on the second day of the Workshop, participants were assigned to new sub-groups to discuss four additional topics:

- Requirements of the Draft Protocol for Facilities and Equipment
- Contingency Planning for Different Outcomes of the Draft Protocol
- Personnel Management Considerations in Implementation of the Draft Protocol
- Draft Protocol Implementation Process and Update Concepts

These Sub-groups reported to the plenary session on the morning of the last day of the Workshop.

On the afternoon of the final day of the Workshop, there was a focused plenary discussion on what areas of research and development are necessary to adequately design the SRF and facilitate the implementation of the final protocol; an outline of the topics discussed in the plenary discussion appears in this document beginning on page 68.

This Working Draft Protocol will now undergo a thorough review and refinement process, the first step of which is a review by the Oversight and Review Committee (see Appendix D3, page 145) currently scheduled to occur in November 2001.[17] Subsequent to the scheduled review and any required amendments/modifications, the Final Version of the Draft Protocol will be issued as a stand-alone document. That document will be circulated for further study, approvals by outside entities, and for use by the Mars Sample Handling Project and others in the design of a sample receiving facility and in the development of an eventual final protocol to be applied to returned martian samples.

17. The Working Draft Protocol included in this report represents an early stage in the development of the final protocol, and is reported here to document this stage in the process. The ORC review of the Working Draft Protocol was completed in November 2001; the Final Version of the Draft Protocol *[Rummel et al. 2002, in press]*, which incorporates the comments from the ORC, is expected to be published in October 2002.

SUB-GROUP SUMMARY REPORTS

Background Information on Workshop 4 and the Sub-Group Summaries

Prior to Workshop 4, materials developed in all the earlier Workshops in the MSHP Series were compiled into a first complete draft of the protocol; which was subsequently designated the "Penultimate Working Draft Protocol."[18]

The stated objective of Workshop 4 was to finalize the Penultimate Working Draft Protocol (PWDP). Each Workshop 4 Sub-group was provided with a copy of the PWDP and given specific assignments. Sub-groups 1, 2, and 3 were each asked to examine specific sections of the PWDP, to refine wording and content, and to identify present limitations and address pending issues;[19] Sub-groups 4 – 8 were asked to address issues not previously covered in the PWDP.

The "Working Draft Protocol," found in this report beginning on page 71, is the result of incorporating the accepted comments and changes from the Workshop 4 Sub-groups into the PWDP. However, not all of the recommendations or comments of the Workshop 4 Sub-groups were accepted for incorporation into the Working Draft Protocol. Therefore, the individual reports from each Sub-group are included here, in their entirety, to provide a complete record of the Workshop 4 deliberations (as a result, there is a fair amount of redundancy in this document).

Sub-Group 1: Review and Assess the Draft Protocol for Physical/Chemical Testing

Charter

The charter of Sub-group 1 was to "Review, assess, and adjust the Penultimate Working Draft Protocol for sample container processing, sample preparation, and physical/chemical analyses: Does the Draft Protocol adequately provide for planetary protection 'containment,' handling, and analysis requirements to protect the Earth, as well as for the requirements to ensure the scientific value of the sample? Can data about the sample be provided in a timely fashion to support the life-detection and Biohazard testing steps of the Draft Protocol, as well as to support sample preservation and curation considerations? Which analyses need to be done in containment either within the primary containment facility or outside of containment using sealed containers? Which analyses can be done outside of containment on samples subjected to a sterilizing process involving heat, radiation, etc., or a combination of these agents, to ensure they are safe for analyses outside of containment?"

Sub-Group 1 Members

Treiman, Alan H. (U.S. Co-Chairperson)
Counil, Jean-Louis (French Co-Chairperson)
Allen, Carlton C.
Allton, Judith H.
Bibring, Jean-Pierre
Collins, Mary E.

18. See the table in the Preface for a listing of the various versions of the Draft Protocol.

19. All changes described in the Sub-group reports refer to changes to be made to the Penultimate Working Draft Protocol.

DeVincenzi, Donald L.
Edelson, Martin C.
Garvin, James
Holland, Heinrich D.
Johnson, Dale W.
Manhes, Gérard
Mills, Aaron L.

Sub-group 1 focused its deliberations on refining those portions of the Penultimate Working Draft Protocol that involved steps related to Physical and Chemical (P/C) processing of returned martian sample materials. P/C processing includes actions affecting the returned samples between the time the Sample Return Canister (SRC) arrives in the Sample Receiving Facility (SRF) and the time that sample aliquots are apportioned for Life Detection and Biohazard tests. P/C processing should include only those actions required in support of planetary protection and future sample utilization. The details of the proposed P/C processing, which draws heavily from protocols proposed or used by others,[20] is outlined in Figure SG1-1,[21] to which the following explanatory sections below are keyed.

Principles: The selected steps and investigations in the P/C processing tracks are motivated by the following principles as functions of the SRF: know what the returned samples are, preserve sample integrity, document everything, anticipate that different types of samples (e.g., gases, fines, rocks and cores) require different treatment, recognize that all data obtained in the P/C processing must serve later scientific investigations, use the minimum sample possible, and provide real-time guidance and adjustment to the process. These principles, initially outlined by the report of the Mars Sample Handling and Requirements Panel (MSHARP) *[Carr et al., 1999],* have been endorsed by all the Mars Sample Handling Protocol Workshops *[Race and Rummel, 2000; Race et al., 2001a; Bruch et al., 2001; Race et al., 2001b].*

The first two principles (know the sample; preserve sample integrity) are, to some extent, inconsistent because every characterization method or action on the returned samples will affect them in some way. This inconsistency has been addressed in two ways. First, all initial characterization procedures in P/C processing are nominally non-contact and non-destructive – all the sample mass remains in the same physical and chemical state after each analysis. Second, most of the returned sample is subjected to only minimal investigations, while only a representative portion of the sample is subjected to more specific (and potentially sample-altering) analyses. The P/C processing and screening methods, except for weighing, involve sample interactions with electromagnetic radiation, principally near-visible wavelengths (near ultraviolet, visible, and near infrared). Several methods use X-rays to probe the samples, but it was recognized that X-rays can (at some dosages) affect biological/organic systems.

20. The protocol shown in Figure SG1-1 is based on the framework previously developed by sub-groups at the first Workshop in this Series *[Race and Rummel, 2000],* and on an earlier report by MSHARP *[Carr et al., 1999],* which are in turn based on the protocols developed at Johnson Space Center for handling and processing of Apollo lunar samples, Antarctic meteorites, and cosmic dust. Over the course of the MSHP Workshop Series, material suggested by various Sub-groups was incorporated into what became the Penultimate Working Draft Protocol; the PWDP is, in general, consistent with the requirements and conditions set forth by the Space Studies Board *[SSB, 1997],* MSHARP Committee *[Carr, 1999],* an earlier workshop on sample quarantine protocols *[DeVincenzi et al., 1999],* and CAPTEM *[Neal, 2000].*

21. Figure "SG1-1" indicates the first figure of Sub-group 1.

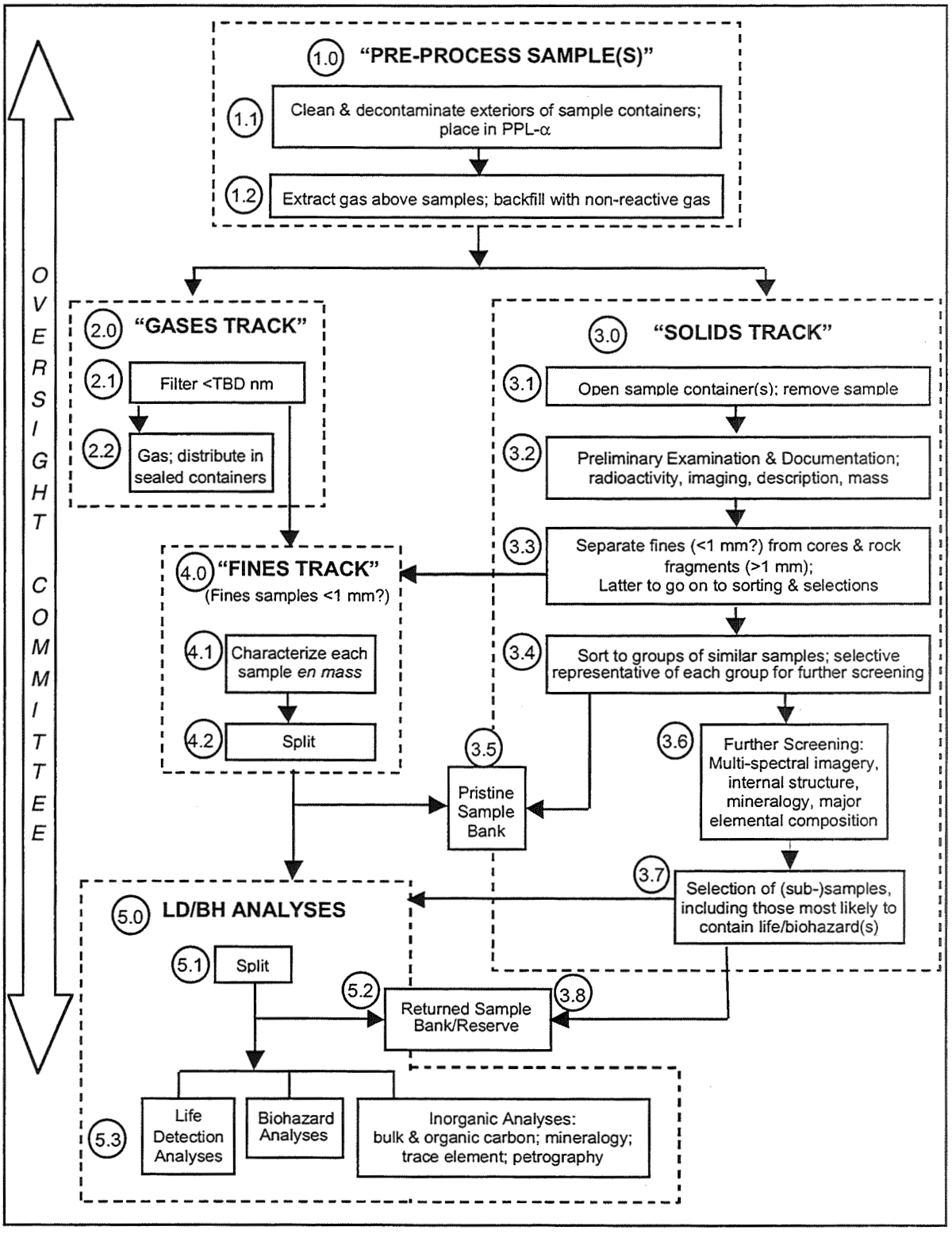

Figure SG1-1. The Physical/Chemical Analyses will occur in four sequential stages leading into the Life Detection and Biohazard Testing.
(The numbers in circles correspond to numbered paragraphs in the text).

The first two principles (know the sample; preserve sample integrity) are, to some extent, inconsistent because every characterization method or action on the returned samples will affect them in some way. This inconsistency has been addressed in two ways. First, all initial characterization procedures in P/C processing are nominally non-contact and non-destructive – all the sample mass remains in the same physical and chemical state after each analysis. Second, most of the returned sample is subjected to only minimal investigations, while only a representative portion of the sample is subjected to more specific (and potentially sample-altering) analyses. The P/C processing and screening methods, except for weighing, involve sample interactions with electromagnetic radiation, principally near-visible wavelengths (near ultraviolet, visible, and near infrared). Several methods use X-rays to probe the samples, but it was recognized that X-rays can (at some dosages) affect biological/organic systems.

This Working Draft Protocol attempts a compromise between the desire to affect only a small proportion of the returned sample by planetary protection testing, and the need to assure safety by testing all portions of all samples. A range of strategies have been advocated to deal with the sample testing issue, from "characterize everything with all available non-destructive methods," to "store most of the sample uncharacterized, and do only the minimum with the rest" (see discussions in *Carr et al., 1999, p. 37; Race and Rummel, 2000, p. 18; Race et al., 2001a, p. 35; Race et al., 2001b, p. 34*). Here it is stipulated that it will be essential to examine all the returned material in at least a minimal fashion to: confirm spacecraft operations in sample transfer from Mars to the Sample Return Canister (SRC); correlate returned samples with documentation made on Mars; and provide enough data to make informed choices about samples for LD/BH analyses. Examining all returned materials in at least a minimal fashion will help avoid a worst case scenario where an obviously biogenic sample could be stored unexamined and only discovered after nominal LD/BH tests were completed.

Documentation: All treatment and actions with the returned samples need to be documented fully. Without a high level of documentation, it would be impossible to establish which samples are representative or particularly interesting, and to indicate what had been done to which sample during processing.

Different Samples: It was clear that the different types of samples will require different processing techniques. Gases and bulk fines samples are expected to be inherently homogeneous to some level, and will require only minimal processing to derive characteristic and representative samples. However, solid materials are anticipated to be potentially heterogeneous and will require more extensive study and real-time decisions about processing.

Minimum Sample Mass: The amount and size of returned Mars samples will be small, and it will be desirable to subject sample materials to a great range of biological, physical, and chemical tests. Thus, by necessity, each test on a returned sample must use the minimum mass consistent with achieving the scientific goal of the test.

Real-Time Adjustments – Oversight Committee: Provisions must be made to adjust the P/C processes in response to changing technology and mission specifics, to monitor the processes in progress, and to adjust them in real time to fit the actual returned samples *[Carr et al., 1999, pp. 7, 9]*. This current Draft Protocol is being written thirteen years before nominal return of Mars samples to Earth. We do not know the spacecraft configuration, the types of martian samples that will be

collected, their return configuration, and the exact nature of planetary protection measures. Similarly, we cannot anticipate the advances in instrumentation and analytical methods that are likely between now and the time of sample return.

It is likely that the returned samples will not be exactly as we imagine them now, and may include materials that are complex (e.g., breccias) or unusual (e.g., a possible stromatolite fossil). Treatment of these types of samples would be sample-specific, and cannot be defined in advance. Thus, there must be a mechanism like a Scientific Oversight Committee to adjust the Protocol to fit the samples.

Assumptions: In preparing the P/C portion of the Draft Protocol, we have assumed the mission profile and constraints outlined in the initial Workshop of this Series *[Race and Rummel, 2000].* It is worth reiterating a few of the key assumptions with particular relevance to physical chemical processing: the SRCs will be received at the SRF intact and with no breaches of containment; the returned samples will include gas, fines material (bulk regolith), and solids; and total sample mass is expected to be approximately 500 to 1000 grams.

Overview of Physical/Chemical Processing: Physical and chemical processing are the priority actions taken with the returned Mars samples between arrival of the SRC at the SRF and initial examination for Life Detection of fines and solids. These anticipated steps in P/C processing are shown schematically in Figure SG1-1 which is based on portions of Figures 6-2 and 6-3 of *Carr et al., (1999),* Figure 1 on page 18 of *Race and Rummel (2000),* and the narrative of *Race et al., (2001a).* The numeric annotations in Figure SG1-1 refer to similarly numbered sections of text below, which elaborates on the proposed P/C processing in narrative form.

P/C processing can be divided into three phases in roughly sequential order:

- Initial pre-processing, before preliminary examination of the samples;
- Preliminary examination and screening of gas, fines, and solids, to permit informed choices about samples for later detailed testing, banking or curation; and
- Sub-division of those samples selected for biohazard and Life Detection tests.

Following P/C processing, Life Detection and Biohazard testing will begin. Those processes may require information developed during preliminary examination and screening, and may also require subsequent more detailed information of a physical or chemical nature. Analyses to obtain these latter data are supplemental to the P/C processing and are not included here.

The steps of preliminary examination and screening were judged different for three types of samples: gases; homogeneous particulate samples; and inherently inhomogeneous samples like rocks, rock cores, and regolith cores. Each of these sample types will follow a different track through preliminary examination and screening as described in the text below and shown on Figure SG1-1 as the *'Gases Track,' 'Solids Track,' and 'Fines Track.'*

Pre-processing Samples

- *1.0 Pre-Processing Steps:* Pre-processing steps outlined here are those between arrival of the SRC at the SRF, and initial examination of gas, fines and solids. Preprocessing steps refer to

cleaning and decontaminating the exterior of any containers holding samples, as well as the initial steps in each of the gases-, fines-, and solids-tracks involving opening of containers and removal of samples.

- *1.1 Clean and Decontaminate Exterior of SRC:* It is imperative that the exterior of the sample return containers or vessel(s) carry no terrestrial microorganisms and are organically clean. (It is assumed that the exterior of the SRC is not contaminated with martian materials.) If these states are not achieved, all subsequent analyses for life or biohazard are severely compromised. The actual methods of cleaning and decontamination are to be determined. An interesting new method for consideration is laser ablation of the SRC exterior.

  Procedures for opening sample containers are mission specific, as to number, types, and contents of containers. At a minimum, we assume that some solid materials with surrounding gas will be in the container(s). It is recommended that the gas be extracted for separate treatment, and that the solid samples be contained thereafter in an inert gas like dry nitrogen.

- *1.2 Extract Head Gas and Back-fill:* The returned solid samples will arrive on Earth with some gas surrounding them. Presumably, this "head gas" would consist originally of martian atmosphere. By the time of arrival on Earth, the gas might have been affected by chemical and physical reactions with the solids (rock and soil), by out-gassing from the solids (especially if the temperature rises above 25°C during return), and possibly by biological activity in the sample. Thus, this gas may contain information important to understanding the thermal, chemical, and biological histories of the solid returned samples. Therefore, extraction and analysis of the head gas is a high priority.

  In this step of pre-processing, the head gas would be extracted from the SRC, and the SRC back-filled with a chemically un-reactive gas to ambient "room" pressure. Exact procedures for extraction and back-filling will depend on the SRC design and construction, but might (for instance) include puncturing the SRC at an intentional thin point, extracting the head gas to a pre-determined vacuum pressure, and refilling the SRC with dry clean N_2 gas. The extracted head gas would be processed as below (see 2.0 – 2.2).

  Three issues related to gases were identified for further consideration and possible research: 1) the effects of vacuum and non-martian gas on the chemical properties of the sample; 2) the effects of vacuum and non-martian gas on any live martian biota; and 3) the effects of extraction on gas isotope ratios.

  For the first issue, experience with curation of the Apollo lunar samples has shown that few geochemical and other inorganic investigations are materially affected by holding and processing the samples in dry N_2 gas at 1 bar. Of course, the lunar samples originated at hard vacuum on the Moon. It is not clear, however, what changes might be wrought on returned Mars samples (possibly containing clays or other hydrous materials) by vacuum pumping and then by immersion in dry N_2 gas; this is an area for research.

  For the second issue, there is reason to want the returned solid samples to be treated under atmosphere as near to martian as possible – both to preserve key geochemical signatures *[Neal, 2000, p. 22,492ff]* and to maintain potential micro-organisms in their native environment. No one

knows whether live martian organisms could be killed by removal of 0.006 bars of CO_2 and then immersion in one bar of N_2 and there may not be comparable terrestrial biota to test. The samples will eventually be subjected to higher pressures, merely because the biota of BH tests would not survive in martian atmosphere. On the other hand, there are serious problems in sample handling and geochemistry that would be caused by immersing the samples in a model martian atmosphere. Sample handling and LD/BH testing at reduced pressure (the near vacuum of 0.006 bars CO_2) present severe problems. Sample handling under vacuum was attempted during the Apollo program with lunar samples, and was found to be extremely difficult, expensive and contaminating (e.g., mercury or oil from vacuum pumps). Similarly, backfill with a relatively reactive gas like CO_2 will change the isotopic nature of the sample. Terrestrial carbon and oxygen will exchange with the sample and compromise biological and geochemical inferences from of these two stable isotope systems.

This is obviously an area of future research. One possible approach would be to backfill the SRC and do sample handling and examination (where possible) under 1 bar of dry N_2 gas with 0.006 bars of CO_2 added. This might satisfy the constraints of easy sample handling and the hope of not killing live martian organisms. However, one wonders if bacteria are affected by unnaturally high partial pressures of N_2.

For the third issue, it is known that the elemental and isotopic ratios of a gas sample can be fractionated during transfer from one reservoir to another. With the head gas in contact with the abundant surface area of the returned samples, fractionation could become a serious potential problem.

Gases Track

- *2.0 Gases Track:* Gas withdrawn from the SRC, the "head gas," will be processed by filtering and subsequently any fines collected will be split for Life Detection and Biohazard testing; the filtered gas would be available relatively rapidly for other investigations *[Race and Rummel, 2000, p. 17].*

- *2.1 Filter to <TBD Nanometer:* After or during removal of the head gas from the SRC, the gas should be filtered to remove particles *[Race and Rummel, 2000, p. 17].* The purpose of filtering the head gas is to remove objects that could reasonably constitute viable organisms or that might present biohazards. The size of objects passing the filter is to be determined (TBD). Sizes suggested by previous sub-groups in this Workshop Series have ranged from <0.5 µm *[Race et al., 2001a, p. 34]* to <0.02 µm *[Race et al., 2001b, p. 27],* both of which are realizable with current technology (currently, some methods are rated to remove particles larger than 0.003µm). It is not clear if filtering could change the chemical or molecular composition of the head gas, for instance by preferential adsorption of heavy noble gases or by catalysis of reactions. This is an area for additional research.

- *2.2 Distribute in Sealed Containers:* The Sub-group recommended that filtered head gas could be released from the SRF and distributed in sealed containers. Unlike the returned solid samples (rock, regolith, etc.), a returned gas sample is only useful for investigation if it is contained. Typically, a gas sample like this would be placed in a glass bulb, which would then be sealed by melting the stem of the bulb. Containment at PPL-α or PPL-β levels seems inherent in this

procedure,[22] and it is recommended that the filtered gas be available for immediate allocation from the SRF without further processing or sterilization.[23]

Solids Track

- *3.0 Solids Track:* After removal and filtering of head gas from the SRC, the remaining returned samples would be solids of various types: regolith samples, rocks, rock cores, soil cores, and fines. The specifics of this solid sample set are to be determined during mission design. These solid samples will be processed through two separate tracks, Solids Track (3.0) and Fines Track (4.0), for basic documentation, further preliminary testing, and selection for subsequent Life Detection and Biohazard tests.

  Some principles of this P/C process are worth restating here. The P/C process is a method to obtain the minimum data needed to adequately characterize the samples and to permit selection of suitable samples for LD/BH tests. The remaining samples would be preserved and made available for subsequent investigations and analyses. The samples will be changed from their original state as little as possible.

  The martian samples will be touched or come in contact with only a limited set of materials under controlled temperature and atmosphere. Pristine lunar samples are touched only by stainless steel, aluminum, and Teflon™; these might also be suitable for returned Mars samples. Neal cites the considerations *[Neal, 2000],* from a geochemical perspective, for choices of materials for sample handling and suggests several types. Whether these materials are appropriate for returned martian samples should be determined through additional research with Mars simulants prior to sample return.

  The temperature of processing is to be determined, and will depend in great part on technical mission constraints. The implicit assumption here has been that temperature of processing will be between 0°C (273K) and ambient (~298K), for which the protocols and experience with the Apollo samples are relevant. On the other hand, it would be important from geochemical and biological perspectives to maintain the returned sample at its ambient martian temperature, ~240K *[Carr et al., 1999; Neal, 2000].* This temperature may not be possible within mission constraints, and there appears to be no compelling reason to process at temperatures significantly below those experienced by the samples during their transit to Earth. It is not clear, at this point, what problems and attendant costs would be associated with sample curation and processing at sub-freezing temperatures.

  The atmosphere of processing, curation, and of back-filling of the SRC is suggested to be 1 bar of un-reactive gas; the composition and pressure of the atmosphere has implications for biological and geochemical testing, and is an area of concern and for future research (see pages 32 and 68 of this report). The following steps implicitly assume that processing and curation will

22. It is assumed that the operation of sealing the gases into the bulbs will be done under appropriate PPL conditions (details TBD).

23. To date, no decisions have been made about when and under what conditions sample materials will be eligible for release from containment at the SRF. Ultimately, it is likely that decisions about what is done with sample materials will be made after review by an appropriate international scientific oversight committee at the SRF in consultation with NASA's Planetary Protection Officer and other responsible officials.

take place under a pure un-reactive gas (such as N_2) at 1 atmosphere of pressure. It is not known whether this gas would present problems to Life Detection and Biohazard testing procedures. It must be recognized that a requirement for processing at low pressure, like the 0.006 atmospheres of the martian surface, would have significant implications for the design and cost of a SRF.

- *3.1 Open SRC and Remove Samples:* The SRC must be opened for retrieval and removal of solid samples. The procedure for opening the SRC and removing the samples are to be determined and will depend entirely on the design of the SRC.

- *3.2 Preliminary Examination and Documentation:* As part of the P/C processing, Preliminary Examination and Documentation includes the minimal investigations deemed absolutely critical for understanding the nature of the returned sample and initial biohazard investigation *[Race and Rummel, 2000, pp. 14, 17; Race et al., 2001a, p. 37].*

  The sole hazard investigation at this time is measurement of sample radioactivity, because some forms of ionizing radiation can penetrate the curation barriers between the returned sample and human processors. The purpose is not to measure abundances of indigenous radioisotopes (e.g., ^{238}U) nor cosmogenic radioactivities (e.g., ^{26}Al), but rather to determine whether radiation levels associated with the samples could pose a threat to workers at the SRF. Hazardous radioactivity can be measured on the bulk returned sample, and need not be measured on individual samples unless the bulk presents a radiation hazard. Only gamma radiation need be detected, as beta and alpha radiation will not penetrate the barriers between the returned samples and human processors. In the opinion of the Workshop attendees, it was extremely unlikely that returned martian samples will present a radiation hazard.

  Imaging provides the first and critical documentation of the returned sample *[Race and Rummel, 2000, p. 17].* Imaging at this stage would serve multiple objectives: verification of mission success; correlation of specific samples with images of them taken on Mars and their sources; documentation of physical effects of transport to Earth (e.g., fracturing, disaggregation), preliminary identification of rock types, and measurement of sample volumes. It is anticipated that the returned samples would be imaged at a high spatial resolution (perhaps ~0.1 millimeter per pixel), in wavelengths TBD (perhaps approximately seven-to-nine wavelengths, with at least three or four in the visible). These data will be critical to understanding the nature of the returned sample and in processing and selection of samples for Life Detection and Biohazard tests.

  Masses of samples should be measured first at this stage, and subsequently whenever a sample is cleaned, split or allocated. Measurement of mass is important as a mission design requirement, for the sample tracking and curation, and for helping allocate suitable samples for LD/BH testing. For instance, it is likely that a mission requirement would be return to Earth of a given mass of martian material, and weighing here will determine if that mission requirement has been fulfilled.

- *3.3 Separate rock fragments and cores from fines:* At this stage of processing, the solid returned sample would be separated into larger and smaller fragments. The former would include drill

cores, whole rocks, and rock fragments or rocklets[24] (equivalent to the Apollo "coarse-fines"). The latter would include unconsolidated regolith, atmospheric dust, and dust generated by coring operations. This separation is necessary because the larger fragments cannot be treated as homogeneous powders, and must be examined individually for Life Detection and Biohazard analysis. It is possible that the regolith samples will include small rocks and rocklets, comparable to the case with the lunar regolith samples returned by the Apollo missions. As with Apollo, the small rocks and rocklets would be separated from the finer material, cataloged, and curated individually throughout subsequent processing and analyses. The cut-off size for rock fragments or rocklets remains to be determined. The standard cut-off size in the soil science community is greater than 2 millimeters. Previous sub-groups in the Workshop Series have suggested sizes ranging from greater than 1 millimeter to greater than 2 millimeters, and even "... greater than several millimeters ..." *[Race et al., 2001a, p. 34; Race and Rummel, 2000, p. 17].* It seems reasonable that decisions about cut-off sizes for different classes of solid materials will be made when the sample is returned and first examined, based on a recommendation of the Oversight Committee (see Appendix D3, page 145).

Given the dusty nature of the martian surface, and the likelihood of dust generated during coring, it is anticipated that the surfaces of cores and rock samples will be coated with fine-grained materials. After separation, preliminary examination, and documentation of the returned solid materials, it will be necessary to remove dust from surfaces of the cores, rocks, and rocklets *[Race et al., 2001b, p. 22].* These fine materials constitute distinct samples of martian material, and will require different processing and curation than the solids (i.e., the fines track). In addition, the fine materials on solids will likely hinder identification and processing of the latter by obscuring their surfaces. Selection of samples for Life Detection and Biohazard assays will require knowledge of the mineralogy, structure, and textures of the samples. The analytical probes available (primarily visual and near-infrared optics) will be unable to operate effectively on dust-covered samples.

The exact method of fines removal are to be determined. Suggested methods have included vacuuming the samples, blowing the dust off, a combination of vacuuming and blowing, and laser desorption. In all these cases, thought needs to be given to how the fines are to be collected after removal. The fines collected from each solid sample would be identified individually, and treated as a separate fines sample within the "fines track," as described in section 4.0 below.

- *3.4 Sort to Groups:* After removal of adhering fines, the solid samples should be sorted into groups of similar materials using visual clues and information from Preliminary Examination data *[Race and Rummel, 2000, p. 17; Race et al., 2001a].* This step assumes that the returned sample will contain several cores and/or multiple millimeter-sized rock fragments ("rocklets"). Criteria for sorting would include size, rock type (including color), grain size, texture, and other readily observable properties. This sorting is an important first step towards selecting representative samples for Life Detection and Biohazard tests *[Race et al., 2001a, p. 26].*

24. The terminology used to refer to small rocky materials has varied from workshop to workshop in this Series. The terms rock fragments, rocklets, and pebbles have been used to identify a general class of solid material that is distinct from fines, larger rocks or rock cores. In addition to determining cut-off sizes at some later date, it will be advisable to use consistent terminology in all parts of the protocol.

- *3.5 Pristine Bank:* Samples and sub-samples that are not chosen at this point for *Further Screening* and/or for Life Detection and Biohazard tests will be stored in a *Pristine Sample Bank [Race and Rummel, 2000, p. 17].* This "bank" will serve as a containment system designed to maintain the physical, chemical, and biological integrity of samples while they await allocation for other analyses at a later date. According to recommendations by CAPTEM, the "bank" should hold the samples under an inert atmosphere at temperatures below 240K *[Neal, 2000].* The pristine solid samples are those that have been affected by no procedures beyond those of preliminary examination, dust removal, and sorting. The pristine bank will serve the critical purpose of preserving a portion of the returned sample for analyses beyond and after the Life Detection and Biohazard assays associated with planetary protection. The pristine bank samples will become the principal resource for all subsequent chemical, geological, physical, and biological analyses on the returned samples.

- *3.6 Further Screening:* At this point, sub-samples of each rock type group sorted previously (see section 3.4 above) would be subjected to additional analyses in support of (and preliminary to) Life Detection and Biohazard tests *[Race and Rummel, 2000, p. 14; Race et al., 2001a, p. 37].* The exact analyses needed are to be determined in conjunction with the detailed LD/BH tests that are also TBD (see *Future Research* on pages 32 and 68). Selected analyses should emphasize non-destructive methods that are not likely to modify or destroy biological molecules and biohazards, and would not be anticipated to kill or weaken live martian organisms. Once they are defined, it will be possible to learn what characteristics of the returned samples would affect or interfere with the tests, and what data are essential prior to the tests. With these data in hand, the *Further Screening* analyses can be tailored to meet the requirements of life and biohazard detection. Given these restrictions and uncertainties, the following screening methods have been suggested.

  Multi-spectral imagery of the samples in visible, near-infrared, and/or thermal infrared light will provide identification of the minerals (inorganic chemical compounds) and presence and distributions of organic matter and water (molecular and bound) in the sample. Raman spectroscopy should be considered here also, with the caveat that samples can experience significant heating during Raman analysis. (For instance, 514.5 nanometer green light from an argon laser is absorbed significantly more than 1064 nanometer infrared light from a Nd:YAG[25] laser. Heating can also be mitigated by distribution of laser power in space and time over the sample). The distributions of minerals on the samples' surfaces will be crucial clues to understanding their internal structures. X-ray diffraction analysis would also be valuable in defining the minerals in the samples (see *Race et al., 2001a*, p. 35ff, for more detail on these methods.)

  It is important to know the internal structures of the samples (especially the larger ones), because biogenic material could reasonably be concentrated in cracks and open spaces (analogous to terrestrial endolithic organisms). Building on the imagery above, tomographic analyses could provide three-dimensional visualizations of the internal structures of the samples. Among tomographic methods, the most developed at present is X-ray tomography. To provide X-ray tomographic maps of density (i.e., continuum absorption of X-rays) now requires only a bench-top instrument. X-ray tomographic maps for individual elements (like carbon) require at present the X-ray intensity of a synchrotron light source, and is likely impractical in this *Further Screening* step.

25. Neodymium-doped:Yttrium Aluminum Garnet laser.

Abundances and distributions of major elements and several minor elements will likely be important for sample selection in Life Detection and Biohazard analyses. It is also possible that abundances of certain elements could produce false positives or negatives on Life Detection and Biohazard tests. A likely method for elemental analysis is X-ray fluorescence, a mature technique used routinely in inorganic geochemistry.

It would be very important at this stage to have bulk analyses for carbon as a guide to sample selection. However, none of the Sample Handling Protocol Workshop sub-groups suggested a non-destructive test for bulk carbon that was sufficiently precise and had low enough detection limits to be useful here. This is an area for future research.

- *3.7 Selection of Sub-samples:* Based on data from the *Further Screening* tests (section 3.6), representative sub-samples will be selected for Life Detection and Biohazard tests. The remaining unselected samples will be stored in the Returned Sample Bank (section 3.8) for future research access. Selected samples will carry forward to the actual Life Detection and Biohazard investigations (section 5.0).

- *3.8 Returned Sample Bank:* The *Returned Sample Bank,* distinct from the *Pristine Sample Bank* (see section 3.5 above), is for storage of samples that have experienced the analysis of *Further Screening,* but have not yet been allocated for Life Detection and Biohazard tests. These returned samples should be labeled and kept distinct from the pristine samples, as the former have had more chance for contamination than the latter.

Fines Track

- *4.0 Fines Track:* Fines samples are those with particle sizes smaller than some to-be-determined limit; the size limit suggested by earlier sub-groups in this Workshop Series was 1 or 2 millimeters *[Race and Rummel, 2000; Race et al., 2001a, 2001b].* In either case, it is anticipated that fines samples will contain so many grains, mixed homogeneously, that it will be readily possible to take representative splits for Life Detection and Biohazard tests. Fines samples may include materials from a variety of sources: material collected as such, like dust from a wind-deposited dune; regolith that has had coarser material removed (see section 3.3 above); dust filtered out of the SRC head gas (see section 2.1 above); or particulates removed from surfaces of rocks or cores (see section 3.3 above).

- *4.1 Characterization:* Characterization of fines samples would be limited to imagery of each bulk fines sample (possibly including multi-spectral imagery) and weighing of each bulk sample *[Race et al., 2001a, p. 35].* There is no need to image or otherwise characterize each individual particle within a bulk fines sample. Only these minimal analyses are needed to document each fine sample at this stage in order to select samples or representative sub-samples for Biohazard and Life Detection assays. Sub-groups at the first Workshop in this Series suggested that each fines sample be subdivided into fragments larger and smaller than 1 millimeter *[Race and Rummel, 2000],* but this suggestion was not pursued by any later Sub-group. It may be an area of needed research.

- *4.2 Split for LD/BH Tests and Banking:* At this point in P/C processing, fines samples would be selected for Life Detection and Biohazard tests, and split into representative aliquots. Some aliquots would be carried forward to Life Detection and Biohazard tests (see section 5.3 below), and some would be reserved in the *'Pristine Sample Bank'* (see section 3.5 above).

  The methods of splitting the fines samples are to be determined. Methods used in typical terrestrial applications (e.g., riffle splitter or coning-and-quartering[26]), may not be appropriate or practical here *[Race et al., 2001a, p. 14]*. First, these methods involve considerable contact between the sample and tools and surfaces, and may be deemed too contaminating. Second, both methods have the potential for considerable loss of sample through embedding in metal surfaces or electrostatic adhesion to metal and plastic surfaces. The electrostatic adhesion problem will be exacerbated in the dry atmosphere of the PPL-α spaces, as has been found with curation of lunar samples. In fact, neither method is now used for splitting lunar fines samples. This is clearly an area for research.

  In this Draft Protocol, it is assumed that a sub-sample of fines is representative, based on confirmation of an adequate splitting method. However, previous sub-groups *[Race et al., 2001, p. 14]* suggested that each fines sample be split into multiple sub-samples and each analyzed for bulk composition and mineralogy (as under *Further Screening*, in section 3.6) to determine whether splits are homogeneous. Further consideration of this issue is needed.

Life Detection and Biohazard Analyses

- *5.0 Samples for Life Detection, Biohazard Analyses:* At this point, samples have been selected for Life Detection and Biohazard tests as well as other P/C analyses

- *5.1 Split into Representative Sub-samples for LD/BH:* The samples selected for Life Detection and Biohazard tests will be split into representative sub-samples at this point. This splitting is necessary to ensure that analyses are performed on similar materials, and so that the results of one test may be reasonably correlated with the results of another. Splits chosen for immediate analysis will proceed to various LD/BH analyses (see section 5.3 below). Some splits will be held in reserve as part of the return sample bank as described in section 5.2. below.

- *5.2 Reserve:* Some splits from section 5.1 will be held in reserve for Life Detection and Biohazard tests, in anticipation of future needs. Should a test fail or require repetition, this reserve material would be available. These reserve splits could reasonably be kept in the *'Return Sample Bank,'* but labeled accordingly.

- *5.3 Parallelism of Tasks:* It is beyond the scope of the P/C procedure to describe the actual operation of Life Detection and Biohazard analyses and supporting inorganic analyses. However, they are included on Figure SG1-1 for completeness. It is anticipated that these three types of tests would be run in parallel, with the results of each influencing the interpretation and course of the other tests *[Carr et al., 1999, p. 9]*.

26. A riffle splitter is a mechanical separation device that is able to split an unconsolidated soil sample into two equal parts that have the same grain size distribution (and presumably composition as the parent sample). Coning-and-quartering is another commonly used separation method (as described in *Maxwell 1968*).

Future Research

In the discussions about physical and chemical processing of the returned martian samples, Sub-group 1 identified several areas where data were not available or could readily be readily obtained without additional research. Each research suggestion discussed below is keyed to the particular narrative text section above where it is called out:

- What analyses and data do the Life Detection and Biohazard analyses require from physical and chemical processing processes? (see sections 3.2, 3.6, and 4.1). These requirements were not available, so the P/C process here reflects informed judgment (mostly from geochemists and geologists) about which analyses would be most useful in LD/BH studies. In particular, it would be very important to know what information about sample characteristics or the particular P/C processing would be important to know for LD/BH purposes (for example, as possible causes of false positives or negatives: to document abundances of specific elements of interest (e.g., arsenic) or minerals (e.g., saponite clay); or to characterize surface reactivity and constituents (e.g., super-oxidants), etc.
- Is there added value in separating each fines sample into grain size separates *[Race and Rummel, 2000, p. 17]*? What additional contamination might be introduced by this procedure? (see section 4.1)
- How can one remove terrestrial contaminants (including organics) from the exterior of the SRC before it enters PPL-α space? Laser ablation surfacing was suggested and should be studied. (see section 1.1)
- How can one effectively remove dust and other fines from the surfaces of rocks and rock cores? (see section 3.3) Three suggestions were vacuuming, blowing with compressed gas, and laser desorption.
- What effects do X-rays have on biological structures and molecules? Several analytical methods involve interaction of X-rays with the samples (e.g., XRD, XRF, XR tomography), and the Sub-group 1 did not know whether these X-ray doses would affect LD/BH analyses. (see section 3.6)
- How can one analyze a bulk sample for trace or ultra-trace quantities of carbon, non-destructively and without anticipated deleterious effects on biological molecules or viable organisms? (see section 3.6)
- Is the chemical composition of the head gas affected by filtration to remove small particles? (see section 2.1)
- How can one produce representative splits of martian dust and fines materials without unacceptable contamination or loss of sample? (see section 5.2)
- How can one confirm that splits of dust or fines material are representative before biohazard and Life Detection analyses, or is such confirmation necessary? (see section 2.2)
- What chemical and physical effects would removal of head gas and replacement with dry nitrogen have on the returned martian samples? (see section 1.2)
- What chemical effects would removal of head gas from the returned sample canister have on the gas itself? (see section 1.2)
- What effects would removal of head gas and replacement with dry nitrogen have on live martian and terrestrial organisms in the returned martian samples? Would these effect be mitigated if samples were curated under dry nitrogen with 0.006 bars of CO_2 gas? (see section 1.2)
- What effects would gas with terrestrial carbon and oxygen isotope ratios have on live martian and returned terrestrial organism in the returned martian sample? Perhaps, would live martian organism ingest the terrestrial carbon and oxygen, and become isotopically indistinguishable from terrestrial organisms? (see section 1.2)

- Using Mars simulants, determine whether materials and conditions recommended by CAPTEM *[Neal, 2000]* are appropriate for handling martian samples. (see section 3.0)
- Petrographic thin sections are enormously valuable in characterizing the minerals, structures, textures and history of a rock. Can petrographic thin sections be produced in a manner consistent with the principles of minimal sample use and minimal contamination of the section material and the remaining sample?

Areas of Concern

Several areas of serious or general concern have been raised during discussions of physical and chemical processing; these issues are significant enough to affect mission design, SRC design, and SRF design:

- The validity and significance of biosafety and LD/BH procedures in the SRF is strongly dependent on sample collection procedures on Mars, and thus on spacecraft and mission design. How can the Biohazard and Life Detection teams have adequate influence on the designs of sample return spacecraft and sample collection procedures?
- What if the return sample container is breached or its seal is compromised? What contingency plans are possible to achieve PPL-α containment and biosafety? (see *'Real-Time Adjustments,'* page 83)
- Is measurement of sample mass important as a preliminary characterization step? Should it be deferred until the *'Further Screening'* step? (sections 3.2 and 3.6)
- How is the head gas to be removed from the SRC without contamination? (section 1.2) Is backfill with non-reactive gas justifiable in terms of possible effects on martian biology? Would it be adequate to backfill with 6 millibars of terrestrial CO_2 and the remainder a non-reactive gas? (section 1.2)
- What should be done if a unique critical sample is smaller than the nominal requirements for LD/BH analyses? (section 3.4 ff)
- What should be done if the requirements for LD/BH testing evolve to consume an inordinate quantity of returned sample, to preclude other biological, organic, and inorganic tests that further NASA's other goals? (section 5.0)
- Although not directly relevant here, concern was expressed that sterilization measures might have significant adverse effects on biochemical analyses outside of PPL containment *[Race and Rummel, 2000].*

Sub-Group 2: Review and Assess the Draft Protocol for Life Detection Testing

Charter

The charter of Sub-group 2 was to "Review, assess, and adjust the Penultimate Working Draft Protocol for Life Detection, considering the following questions: Are data available from the first-tier physical/chemical analyses to support further analyses for Life Detection? Can the Draft Protocol be expected to yield evidence of living organisms within a martian sample? Can terrestrial organisms that might contaminate the sample be detected and identified as such? Can the Draft Protocol enable the detection of life-forms which are not based on Earth-biochemistry, but which have an active metabolism? Which analyses need to be done in containment either within the primary containment facility or outside of containment using sealed containers? Which analyses can be done outside of containment on samples subjected to a sterilizing process, involving heat, radiation, etc., or a combination of these agents, to ensure they are safe for analyses outside of containment?"

Sub-Group 2 Members

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Mustin, Christian (French Co-Chairperson)
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Stabekis, Pericles D.
Voet, Donald
Wainwright, Norman

[Editors' note: Sub-group 2 prepared their report in the form of a list of comments and suggested changes to the PWDP; no attempt was made to re-write their report as prose. Any page numbers or figure/table numbers mentioned in this Sub-group's report refer to pages, figures, and tables in the PWDP.]

Principles

- Start with broadly-defined signatures that cover all known terrestrial life and might cover non-terrestrial life (modify "bio-signatures" p. 24-25, move to beginning): structural, chemical-structural and biosynthetic, isotopic, geochemical
- Most likely scenario for non-terrestrial life involves prebiotic mix similar to early terrestrial; but different evolutionary path: carbon-based, but slightly different building blocks and polymers
- De-emphasize specific focus on non-Earth-based (non-carbon-based) life (delete Table LD1)
- Will we be able to recognize prebiotic chemistry?
- Table LD2 ("Universal Properties of Life") modified: #3 – Life competes for resources, as a result, it replicates, evolves, etc.
- Table LD2: Each category might be found alone (e.g., self-sustaining catalytic system), and as such, could constitute a sign of life (non-terrestrial); it is the <u>combination</u> of all categories together that define <u>life as we know it</u>.

Analytical Methods

- *Emphasize general approach:* broad survey of portion of different sample types for suggestive features – structure, basic chemistry (organic or complex carbon), local inhomogeneities; then, *focussed* examination for polymers, more complex structures
- *Survey mode:* microscopy, broad-band fluorescence scanning, surface scanning/chemistry, tomography (outside SRF?); add isotope release experiments (e.g., Viking)?
- *Focussed mode:* MS approaches, combustion/acid, isotope analysis; add (new) electron microprobe methods for cell scale elemental mapping
- *Need further development of methods:* for characterizing rare complex polymers (see earlier Workshop) and criteria for positive result in assessing complex carbon
- *Survey methods:* may be less sensitive to quantity or location, or less specific; but allow targeting (focussed methods), and most effective use of samples
- *Culture:* likelihood of negative results

- *Expand:* search for geochemical signatures of life, e.g., pigments (photosynthesis), other inorganic chemical anomalies (iron, sulfur, etc.)
- *Add:* search for localized overabundance of discrete subset of related compounds (self-sustaining catalysis)
- *Add:* finding of co-localized, multiple putative organisms increases likelihood that they represent life-forms

Integration, Organization of Methods

- Minimally-destructive methods can guide use of grossly-destructive methods
- Start with samples least likely to contain life (surface fines); if negative, use as blanks, controls for spiking
- Need elaboration of procedures for transportation of samples under PPL-α
- Remember: if identified in a sample, Earth-based life contamination cannot necessarily be assumed to explain all evidence of life found in that sample, especially when using different methods; i.e., both Earth and Mars life may be present
- Controls ... more detail needed (regarding methods, and problems of heterogeneity)
- Sampling spacecraft prior to departure (archive and analyze up-front)
- Return of martian atmosphere in separate but identical container
- Ubiquity of terrestrial life signatures in reagents, etc.; therefore, need negative controls incorporated which involve blank handled in near-identical manner (what is the blank? either treated Earth sample, or ... ?)
- Because we expect many "negative" results, need to determine level of sensitivity, so that result can be described relative to this level
- Exposure of sample surface to PPL-α atmosphere will (unavoidably) cause deposition of particulate matter; therefore, need to analyze this process over time on a "blank" sample

Other Considerations, Comments

- Include commentary on usefulness of replicate analyses after elapsed time in SRF for further evidence of life or bioactivity (changing signatures). Also, value of repeated measurements after perturbation (e.g., altered environmental conditions)
- Add commentary on relative value of specific methods for detecting on-going activity versus inactivity
- Sample "treatment" (sterilization) is likely to eliminate ability to continue Life Detection
- Distinguishing terrestrial from non-terrestrial life: if former is intact or replicating – little problem; but if former is fragmented or evidence is fragmentary, and/or if non-terrestrial life is closely-related to terrestrial, then the task will be challenging
- May not be wise to include a specific time period (90 days) required for preliminary Life Detection

The following are comments by Christian Mustin (Centre de Pédologie Biologique, France) regarding the prioritization of analytical methods for Life Detection, upon which the Sub-group largely agreed. Analytical methods should be arranged in a prioritized order using as criteria the most likely biosignatures or more restricting hypotheses of life occurrences. Moreover, minimally destructive methods (task 1) can guide use of destructive methods (tasks 2 and 3):

- *First task "structural signs of life":* (obvious signs of cellular structure or biomineralization)
  - Non destructive methods, done in containment: microscopy and microspectroscopy (Raman, IR, broad-band fluorescence); 3D tomography, surface scanning/chemistry.
  - Needs referenced database of the characteristics of likely microbial ecosystem chemistry (lithotrophic, endolithic, extremophilic environments) and complex life structures
- *Second task "chemical signs of life":* Chemical species of life (Carbon, rare complex polymers and others chemicals):
  - Destructive methods: LD/MS, carbon analysis, isotopic analysis, solvent extraction
  - Define features of complex chemicals (rare polymers)
  - Measurements done outside the containment: Control of contamination, development of PPL-α sealed container
- *Third task "biochemical and molecular signs of life":* Markers of life (Earth-centric approach – genetic), distinguish terrestrial versus martian life:
  - Molecular methods, amplification techniques, done in containment: PCR, LAL assays, ATP analysis, flow cytometry
  - Extended databases (to be completed): Matches or mismatches with known species; distinguish terrestrial from non-terrestrial life
- *Fourth task "Replication of structures or signs of bioactivity":*
  - Culture under terrestrial or martian conditions: Detect self sustaining catalytic system or self-replicating system, detect structural, chemical and biochemical changes, etc.
  - Inventory media formulation, check compatibility with martian environment (mineralogy, energy and carbon sources)
  - Iterative approach: specific methods for detecting on-going activity versus inactivity could be found in Tasks 1, 2, and/or 3

Controls for Life Detection

Two levels of control (two issues for control) must be defined:

- the first one concerning the integration and the organization of the final protocol and the likely (or unavoidable) contamination of samples during experiments or their transport under PPL-α condition.
- the second, more specific concerning considered methods for the detection of life or of bioactivities

For Integration of Protocol

- Evaluate change in pristine atmospheric sample and estimate "spontaneous" reactivity of sample, by returning martian atmosphere samples in separate containers. Check effect of sealing.
- Development of PPL-α containers designed for outside analyses, including a specific procedure for control of contamination during transport. Need to analyze this process over time on a "blank" sample
- Evaluate adsorption of molecules (high specific surface area), effect of box material, atmosphere changing, terrestrial contaminants
- Evaluate the efficiency of decontamination or sterilization procedures to reduce contaminant load.
- Effect of heat and ionizing radiation treatment ("sterilization") on the chemical properties of samples. Ionizing radiation produces electrons, hydroxyl radical and hydride radical. Each of

these reactive molecules is capable of degrading and altering organic matter (polymers)[27] and surface chemistry of mineral sample (solubility of mineral for instance). Evaluate reactivity of sample (solubility rate).

- Sample "treatment" (sterilization, killing or removal of all viable organisms) is likely to eliminate ability to continue Life Detection

For Detection of Life and Bioactivity

The Life Detection process is an "open box" which will be modified over time (depending on previous analyses).

The choice of blank or control procedures should take into account the low likelihood of detecting life. It should be ordered to take into account a variety of routes of sterilization or inhibition methods to limiting growth of potential living organisms or to reducing bioactivity (change in conditions).

- Validate control procedures and Life Detection methods by the employment of soil simulants and earth microbes living in similar microhabitats
- The complete killing (cidal effect) or destruction of all organisms (i.e., lysis at high energy) is not necessarily required.

Additional Issues

Equivocal Findings: Problems of terrestrial microorganisms evolving on Mars/living relics of earliest life-forms/Earth organisms living in particular conditions (extremophilic). Perhaps, there is little latitude for genes to change significantly, if a self-replicating system maintains itself successfully under such harsh conditions. Matches with an extended genus database.

Sensitivity: Are we able to detect the activity of scarce life? What is the confidence of blank test results? To what degree are the small samples representative of the larger samples?

- Need to determine level of sensitivity of detection methods, if many "negative" results are expected;
- Results can be discussed relative to this level and blanks used to validate.

Culture Strategy: Countless microhabitats (niche, heterogeneous microenvironment) exist on Earth and are responsible for a great metabolic diversity and biodiversity. For instance, mineral surfaces will be of considerable importance as likely life habitats, because nutrients can be adsorbed to them (nutrient levels higher than in bulk solution). We must therefore, learn "to think small and rare" and incorporate these considerations into the protocol methods and processing. A conceivable culture strategy could be:

- Duplicate as closely as possible resources and conditions of the pristine niche;
- Define microhabitats and the bioavaibility of nutrients ("feast or famine"). Use spiked Mars soil simulants.
- Measure the activities of organisms and monitor their effects on the niche. Inventory likely life signatures and ubiquity (chemical changes)

27. A 80 kGy (8 Mrad) dose degrades polymeric molecules (humic acid) in soil.

Comments on Relative Value of Specific Methods for Detecting On-Going Activity Versus Inactivity

Because physico-chemical conditions in a microenvironment can change in terms of both time and space, replicate analyses after elapsed time in BSL-4 containment will be useful for further evidence of life or bioactivity (changing chemical signatures) or repeated measurements after perturbation (e.g., altered environmental conditions). Sample "treatment" (sterilization, killing or removal of all viable organisms) is likely to effect subsequent Life Detection.

The following sections present an elaboration by George Fox (University of Houston, Texas) on comments he made during the Sub-group discussion and upon which the Sub-group largely agreed, concerning the incorporation of concepts on the origins of life in the design of Life Detection methods. Fox noted that the Life Detection Sub-group had focussed much of its effort to date on defining properties of life and then devising experiments to determine if any of the indicator properties are present in a sample. An alternative way of thinking about the problem of Life Detection is to consider how life might have arisen on Mars and/or the Earth. When one does this it clarifies why it is so important to determine not only if there are complex organics but also their composition.

Independent Origins of Martian Life: Existing theories of the origin of life on Earth suggest that life will arise as a consequence of chemical and physical principles anywhere prebiotic carbon compounds accumulate in suitable environments, e.g., water, temperature, etc. in sufficient amounts for sufficient time. Although the precise process for life's origins on the Earth is not known, it is perceived to have been a progression in complexity beginning from an original prebiotic mixture, at some stage involving RNA catalysis, and probably at later stages catalysis by peptides and proteins, ultimately culminating with the first simple organisms that had a metabolism, the ability to replicate and the capability of preserving useful information during the replication process. It is hypothesized here that if a unique carbon-based life system exists on Mars that it arose by similar processes as those which led to the origin of life on the Earth. A critical question for theories of the origin of life is whether the process is primarily deterministic, or perhaps alternatively has stochastic components. In the later case, evolutionary theory suggests it is extremely unlikely that there would be one precise outcome to the origin process, especially if it were to occur in different places with different initial conditions.

There is no clear reason why there should be one and only one series of events that lead from prebiotic chemistry to true life. Thus, when life arises in other environments, e.g., Mars, it is likely to deviate from the path followed on Earth as soon as there is a significant possibility of doing this. In other words, the similarity between life systems with independent origins will to the first approximation depend on the extent the process is unique. In fact, current thinking about the process on Earth suggests that it had many steps and stages with selection and competition acting on molecules and entities early on just as it later acts on the organisms. Therefore, deviations leading to dramatically different outcomes might have occurred at many stages.

This theoretical framework suggests that if martian life were descended from an independent origin that the most likely scenario is that it would likely differ in fundamental biochemical properties such as the choice of fundamental amino acids or nucleotides used, types of lipids, chirality, etc. If divergence occurred much later in the process but still before a true organism had emerged we would expect less fundamental differences such as different choices for the codon assignments (not usage) or the

structure of tRNAs and the ribosome. The primary indicator of past or present life of this type would be to find unusual macromolecular assemblages, e.g., peptides or oligonucleotides with nonstandard amino acids, non-standard bases, non-standard linkages, etc.

A crucial experiment in the absence of obvious indicators (e.g., structural signatures, ability to grow etc.) is to conduct a detailed analysis of complex carbon (if any is found). It will be essential to determine if there is evidence for novel amino acids, peptides containing novel amino acids, chirality effects, etc. However, complex carbon found in meteorites partially fulfills this condition so a positive result would require a comparison with controls to establish that the amount and type of compounds seen is outside the normal range of that found in meteorites (e.g., Lunar samples, and terrestrial samples).

A related but far less likely scenario is that divergence from the origins path was excluded until true organisms existed, with the result being that the organisms would be fundamentally Earth-like. In such an instance, it would likely still be possible to recognize this by sequencing 16S rRNAs. Trees built from these data would likely reveal the martian organism to be as different from Bacteria and Archaea as these Kingdoms are from one another.

Finally a complexity might arise. It is within the scope of imagination that an aberrant composition found in the complex organics might be the consequence of middle to late stage prebiotic chemistry that never got as far as true organisms.

Forward/Backward Contamination: If life is detected directly or by Earth like biochemical patterns in the complex organics, then there are several possibilities. One of these is that life arose independently on only one of the planets (i.e., Earth or Mars) and was transported to the other billions of years ago much as the martian meteorites reached Earth. In this scenario, tests such as 16S rRNA sequencing would again reveal organisms that did not tree with any of the major kingdoms, (genes for novel enzymes not found on the Earth etc.). If the transfer were more recent (hundreds of millions of years), the organisms might tree with particular sub-clusters, but no direct match would be detectable. If however forward contamination associated with the mission itself were to occur, it should be possible to find an exact match.

Non-Carbon Based Life: It is impossible to speculate at this stage how simple non-carbon-based life might arise directly from a prebiotic world (such non-carbon-based-life might arise indirectly from advanced carbon based systems, e.g., descendants of intelligent robots/smart computers, etc.). It is clear however from the agreed properties of life that such an entity would require complex compounds. An important experiment here may be to detect the presence of compounds containing Si, Al, Fe, etc. that are of unusual complexity compared to what is normally found on the Earth, in meteorites or lunar samples. It is not clear to this writer whether instrumentation to do this is available and therefore research might be required for this purpose.

Sub-Group 3: Review and Assess the Draft Protocol for Biohazard Testing

Charter

The charter of Sub-group 3 was to "Review, assess, and adjust the Penultimate Working Draft Protocol for Biohazard testing: Are data available from the first-tier physical/chemical analyses to support the analyses for infectivity/biohazard (especially the presence of toxic materials)? Can the Draft Protocol be expected to yield sufficient evidence to rule out any reasonable doubt over the absence of biohazard in the samples? Will the Draft Protocol allow for a broad-spectrum of challenges with the sample material that can reasonable be expected to show a response if the sample displays infectivity or a similar biohazard? Can the Draft Protocol results provide indications of the potential for chronic effects that should be assessed separately? Can terrestrial organisms that might contaminate the sample be detected and identified as such if a biohazard is detected? Which analyses need to be done in the primary containment facility, and which can/should be done outside of the primary containment facility using samples selected and shipped to another containment laboratory or kept in sealed containers?"

Sub-Group 3 Members

Richmond, Jonathan (U.S. Co-Chairperson)
Sourdive, David J.D. (French Co-Chairperson)
Battista, John
Bielitzki, Joseph
Chamberlain, Virginia
Fishbein, William N.
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Fultz, Patricia
Gabriel, Dean W.
Grange, Jacques
Khan, Ali S.
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McSweegan, Edward
Pardee, Arthur B.
Schad, Jack
Stanbridge, Eric J.
Viso, Michel

Sub-group 3 examined the PWDP for format, wording, content, and present limitations related to Biohazard testing. Since many key issues had already been addressed during previous Workshops, Sub-group 3 focused on perfecting parts that had already been substantially worked through, and on identifying and addressing pending issues. The changes described below refer to changes to be made to the PWDP.

Simple Modifications to the Penultimate Working Draft Protocol

Definitions and Terminology: Sub-group 3 started by addressing the issue of definitions and terminology used in sections relating to Biohazard testing. Several recommendations were made to clarify terminology in particular sections, and to suggest 'global' changes throughout the PWDP and

all subsequent documents on the topic. Sub-group 3 recommended the following changes to specific words or phrases:

- Change "replicating" to "replicating or amplifiable by a biological system." This new terminology will not limit biohazard to "living" entities which, depending on the perception of the dogma, may not include genuine biohazard such as viruses.
- Change "adverse effect" to "significant alteration." This new terminology will not limit biohazards to entities that are immediately or acutely toxic.
- Change "non-biohazardous to humans" to "non-biohazardous" (in the paragraph "*If the initial Biohazard tests and Life Detection tests are all negative, it would be appropriate to conduct subsequent tests under less strict containment conditions once sample materials have been shown to be non-biohazardous to humans.*"). This new terminology still includes biohazard to ecosystems.

COMPLEX Report: Sub-group 3 also suggested a change in the location within the PWDP for recommendations by the Committee on Planetary and Lunar Exploration (COMPLEX). The Conclusions from the COMPLEX report *[SSB 2002]*[28] should be taken out of the body of the PWDP text on pages 7 and 8. The information from the COMPLEX report should be placed in an appendix and referred to at the beginning of page 7.

Sequence of Tests: Sub-group 3 discussed the section of the PWDP on "Sequence of Tests" and agreed on two important conclusions. First, any particular test proposed today will likely be unacceptably obsolete at the time that samples are returned and the final protocol is implemented. Therefore no tests should be dictated ten years ahead. Second, the criteria for selecting tests, which was discussed in Workshop 2 of this series *[Race et al., 2001a]*, will still be valid even with improvements. Thus, Sub-group 3 made the following recommendations:

- The criteria for choosing tests (the 7 points from page 25 of the final report of Workshop 2, *[Race et al., 2001a]*) should be included at the top of page 30.
- Change the sentence on page 30 "The following specific initial tests were recommended by Workshop 2 to be included in the Draft Protocol." to read "The following specific initial tests were *suggested* by Workshop 2 to be included in the protocol *should it be carried out today.*" This change of wording in the Draft Protocol will make it consistent with the actual charter of the Biohazard Sub-group during Workshop 2.

Levels of Containment and Number of Facilities: Sub-group 3 discussed the issue of levels of containment throughout the sessions. For the sake of clarification, and in order to keep a coherent set of definitions throughout the documents (and all further documents), Sub-group 3 made the following recommendations:

- Include the PPLs matrix from page 45 of the Workshop 2 final report in the Draft Protocol, before Figure BH1.[29]
- Explicitly define the levels of cleanliness associated with the different levels of containment envisaged.

28. At the time of Workshop 4, the COMPLEX report was still 'in press' but advance copies were available to the participants for reference.

29. The final version of the figure referred to here as "BH1" appears as Figure WDP-4 on page 109 of this document.

- Change the term "BSL-3" to "BSL-3-Ag."[30] Level 3 laboratories abide by different standards within the U.S. and Europe. The actual level 3 standard required for Mars samples is equivalent to U.S. BSL-3-Ag.
- Include a clear statement that only the primary Sample Receiving Facility (SRF) will have to provide PPL-α conditions. If facilities beyond the SRF are used as part of the Draft Protocol testing, these other facilities will be certified for conducting studies and tests at PPL-β, PPL-γ, and PPL-δ conditions.
- Sub-group 3 discussed the level of containment and cleanliness required for each kind or stage of testing in the Draft Protocol, and recommended that BSL-3-Ag facilities should be built around large instruments, rather than miniaturizing instruments to fit into a pre-existing lab. Both the cost and the feasibility of transforming instrumentation are unfavorable relative to the well-known and well-documented procedure of upgrading facilities to biosafety level 3.
- Modify flow chart BH1 in the Draft Protocol as follows:
  – Move "*in vivo* tests" and "*in vitro* cells" upwards into the PPL zone.
  – Take out the "gas" text box
- Mobile containers should be certified at the appropriate PPL levels (as opposed to BSL requirements exclusively) to allow transport of samples. Sub-group 3 noted that procedures for routine transportation of biohazardous material(s) are already used by BSL-4 and BSL-3-Ag facilities.

Irradiation of Samples: Sub-group 3 discussed the issue of possible irradiation of samples, although it is not part of Biohazard testing *per se*. Two divergent points of view emerged during the discussion.

Some Sub-group 3 members argued that even in absence of evidence of biohazard, samples should be gamma irradiated at substantial doses (equivalent or higher to those used for present day BSL-4 sample release) prior to gradual de-containment and release.

Those with a second perspective argued that, if Life Detection and Biohazard test results allow the conclusion that samples are safe enough for release, there is nothing in them that can be made safer by irradiation. Resorting to such treatment will unnecessarily destroy information and shed suspicion on the entire Final Protocol. In addition, the word "sterilization" should be STRICTLY reserved for the situation where a genuine organism can actually be grown prior to irradiation and can demonstrated as no longer active after such treatment. In all other instances, this wording ("sterilization") should be avoided because it implies there is conclusive evidence of biohazard inactivation, which may be misleading or provide a false sense of safety in communications with decision makers and the public.

Despite lengthy discussion, Sub-group 3 did not reach any consensus and could not make any recommendations regarding irradiation or sterilization of samples.

Pending Issues to be Addressed and Added to the Draft Protocol

There are three issues to be addressed and added to the PWDP in order to finalize it: Sub-sampling, Decision Making, and Communication of Results.

30. For the most current definition of BSL-3-Ag, consult the web site of the US Department of Agriculture <http://www.afm.ars.usda.gov/ppweb/242-01m.htm>

Sub-sampling: Sub-group 3 noted that the difficult issue of sub-sampling procedures for Biohazard testing, which although mentioned repeatedly in earlier reports, was not addressed in detail in the PWDP. In their discussions, the Sub-group attempted to identify methodological approaches that could provide reasonable statistical relevance for tests performed on sub-samples. Sub-group 3 recommended that this crucial issue should be addressed more thoroughly in the PWDP.

Sub-group 3 concluded that if no characterization of samples is provided, only random sub-sampling can be performed and for some samples (rocks, pebbles, etc.) it may be questionable, and release may never be recommended.

Since fines can be considered "homogeneous" and can be sub-sampled as one category in a statistically relevant way, Sub-group 3 recommended that Biohazard testing be initiated using sample materials in the 'fines' category.

Decision Making: Sub-group 3 also noted that in the PWDP, no procedure was described for data interpretation or decision making, both of which are crucially important issues. It is likely that test results will not lead to unanimous consensus in all instances. Sub-group 3 emphasized the importance of following a strict scientific procedure in reaching conclusions as well as the need to involve selected, multidisciplinary experts and expert groups in the decision-making. The Sub-group noted the importance of addressing the overall decision making process as well as procedures for drawing conclusions, certifying results, and deciding that samples are safe enough to be released to lower containment levels.

Sub-group 3 recommended adding to the PWDP sections on decision-making and choice of expert panels in charge of decisions associated with Biohazard testing. At a minimum, the principles of decision-making should be outlined in the final protocol document.

Communication of Results: Since Biohazard testing is unlikely to provide certainty about returned samples, it will be important to address publicly the subject of acceptable risks and benefits associated with any release of martian materials. Sub-group 3 emphasized the importance of developing a strong public communication plan far in advance of sample return to keep both the public and the scientific community informed of results during Life Detection and Biohazard testing. In recognition of the inherent problems associated with dissemination of partial results or inconclusive observations, Sub-group 3 also noted the need for procedures and criteria (e.g., level of certainty, consensus or majority, etc.) for determining how observations and data will be designated as 'results' suitable for formal announcement.

A public communication plan should be developed well in advance of sample return to keep both the public and the scientific community informed of results during Life Detection and Biohazard testing. In addition, specific plans for a formal/official 'Announcement of Results' should be included as part of the Draft Protocol.

Sub-Group 4: Environmental and Health Monitoring and Safety Issues

Charter

The charter of Sub-group 4 focused on "Environmental and health monitoring and safety issues to be considered in the Penultimate Working Draft Protocol: What sort of monitoring capabilities both within and outside of the containment area should be required to ensure the health and safety of the human workers in the primary receiving laboratory and any secondary facilities? What sort of capabilities should be required to ensure the adequacy of containment and the safety of the environment outside the primary receiving laboratory? Even if no biohazard is found in the samples, and they do contain non-biohazardous toxics or radioactive material – what measures should be required or recommended to ensure the safety of those working with samples that are analyzed outside of containment (both in the case of samples subjected to a sterilizing process to ensure they are safe for analyses outside of containment, and in the case of samples that have been released for scientific study during or after sample recovery)?"

Sub-Group 4 Members

Cambon-Thomsen, Anne (French Co-Chairperson)
Leonard, Debra (U.S. Co-Chairperson)
Crissman, Harry
Daly, Michael
Dawson, Sandy
Debus, Andre
Emmett, Edward
Race, Margaret
Rummel, John
Ryan, Margaret
Scannon, Patrick
Vasil, Indra

In essence, the charter for Sub-group 4 was to determine methods for monitoring the health and safety of the personnel of the SRF and the environment in and around the SRF, as well as at secondary sites, if any, in implementing the final protocol. The Sub-group considered monitoring over time, beginning prior to the arrival of Mars samples, during work on the Mars samples at the SRF and at secondary sites, and considered how long to continue monitoring.

Assumptions

- The real risks associated with the Mars samples are unknown.
- The greatest potential risk is biological and includes "life as we don't know it."
- The potential exposure in the SRF will be of a small group of trained professionals until more information about the nature of the specimens is available.
- A high level of security for the SRF and the samples will be maintained as part of the PPL designation.

Recommended Principles for Development of Monitoring Program for SRF

Whenever possible, the monitoring plan should use existing regulations and standards. Since international teams will be working on the Mars samples, the regulatory standards from all participating countries should be reviewed and considered when developing the final monitoring plan. During the consideration of existing regulatory standards, the strictest standards, as appropriate for the anticipated hazards, should apply. Exemptions from existing regulations may be necessary because of, for example, differences in the protection of medical information between the participating countries. Achieving the maximum protection possible from the anticipated hazards for the individuals involved should be the leading principle for personnel monitoring and safety. Because of the unique nature of the potential hazards, additional controls than routinely used for hazard monitoring may be required. The monitoring plan should be designed to maintain a balance between the estimated risks to individuals, the environment or the general population and the personal impositions of the monitoring program. The monitoring plan should allow for cross-correlation of the data from the Life Detection and Biohazard testing of samples with the data from the monitoring of the SRF personnel and environment and allow for modification of either set of procedures.

Potential Hazards Considered in the Discussions

Five categories of potential hazards were considered: physical hazards, potential chemical hazards from non-biological toxins, biological hazards; failure or breach of containment; and psychological hazards. The physical hazards include hazards associated with equipment within the SRF labs and radiation from the Mars samples (which is expected to be negligible). The potential chemical hazards are predominantly from non-biological toxins. The biological hazards will clearly be the most difficult to monitor. The psychological hazards are those that may arise for personnel working under PPL conditions. Finally, monitoring of containment is a significant part of the monitoring program. Recommendations for monitoring for all hazards are as follows:

1. *Physical Hazard Monitoring (Radiation and Equipment):* Radiation is a standard hazard with well-established protocols for protection, handling and monitoring. To confirm the expectation that the Mars samples will not present a radioactivity hazard, a radioactivity measurement should be one of the initial measurements in the physical/chemical assessments. The measurement should be at a level appropriate to assess for a biohazard risk, and not to assess the absolute level of radioactivity present. Therefore, standard radiation safety protocols should be in place prior to the arrival of the Mars samples. If the radioactivity level does not represent a biohazard, then the monitoring for radioactivity can be discontinued, unless required for equipment used in the SRF. If a biohazardous level of radioactivity were detected in the Mars samples, then the radioactivity monitoring program would be continued. Other risks from equipment or facilities can be addressed by standard procedures of training and maintenance.

2. *Chemical Hazard Monitoring:* A chemical hazard from the Mars samples is most likely from non-biological, non-replicating toxins, if present. The presence of toxins will be assessed early in physical/chemical testing. If an unusual substance or chemical is identified, specific monitoring methods for that substance can be designed and the substance could then also be used as a marker for breach of containment monitoring.

3. *Monitoring of Containment:* Standard methods for monitoring of containment in BSL facilities can be adapted for use in PPL facilities and can be used to define a breach of containment or potential personnel exposure. If a breach occurs within the SRF, the breach can be corrected by standard procedures and personnel exposures can be assessed. If a breach occurs to the environment outside the SRF, a procedure should be developed to assess for possible environmental and/or human consequences. Procedures for handling a breach to the outside due to differing causes (e.g., leak, disaster, security breach, etc.) should be considered in the development of the plans for handling a breach.

4. *Monitoring of the Environment:*

   - *Before Mars Sample Arrival:* A baseline assessment of the environment around the SRF should be made prior to the arrival of the Mars samples. The assessment should survey the pre-existing environmental conditions, and include an assessment of the water, air, flora, and fauna. This type of survey will likely be accomplished as part of the Environmental Impact Statement required prior to building SRF. During the baseline survey, sentinel species (microbes, insects, plants, animals) can be identified to use for monitoring for environmental changes. Consideration should be given to including some of the same organisms in Biohazard Testing. In case of noted changes in the environment around the SRF after arrival of the Mars samples, the Biohazard Testing results could assist in determining if the changes were related to the Mars samples.

   - *During Mars Sample Handling at the SRF:* Once the Mars samples are in the SRF, environmental monitoring could focus on the identified sentinel species and any novel components of the Mars samples, if identified. It may be useful also to track and record the weather conditions in area of SRF, for correlation in case of reports of a breach to the outside or any unusual events. If changes in the environment are noted on routine monitoring, assess if a breach has occurred. If a breach did occur, the breach procedures should be followed to reestablish containment and clean up any contamination. If changes in the environment are noted and a breach did not occur, assist with investigating the cause for the environmental change to establish that it either is or is not related to the SRF and Mars samples.

   - *After Completion of Life Detection/Biohazard Testing:* The level of continued environmental monitoring required should be reassessed based on the conclusions of the Mars sample testing protocols. Consideration should be given to maintaining the security and containment within SRF for assuring the proper curation of the Mars samples.

5. *Monitoring of the SRF Personnel:*

   - *Before Mars Sample Arrival:* A process of certification for people who will work in the SRF should be developed that includes education about procedures and risks for employment, security clearance, and medical examinations and tests. Clear inclusion and exclusion criteria based on the results of the certification procedures should be developed prior to the hiring of personnel.

     Baseline medical evaluations of personnel should use the existing medical evaluation standards appropriate at the time the evaluations are performed. Since the SRF will be functional for a period of time prior to the arrival of the Mars samples, monitoring before the

arrival of the Mars samples could include several evaluations (a period of two years was proposed). Recommended baseline evaluations include a medical history, physical examination, tests on the person (e.g., chest X-ray), and tests on samples from the person (e.g., blood and urine). All testing should be as non-invasive as possible and maintain a balance between estimated risks from the Mars samples and the risks associated with the tests. Specimens should also be archived for future comparison, if needed, and may include serum, lymphocytes, semen and/or hair. In addition neuro-psychological evaluations using standard testing techniques with well-established interpretation methods should be administered. Symptom data should be obtained using standardized instruments such as the Millennium Cohort survey (USA) or the GAZEL Cohort survey (France).[31]

- *During Mars Sample Handling at the SRF:* A schedule for regular evaluations of personnel should be established, using the same evaluation methods as used for the baseline data collection. Procedures for standard medical management of personnel illnesses should be available either on site or with adequate transportation to a medical facility, as needed. Intervention should be correlated with an identified or risk of exposure to the Mars samples. If an exposure occurs and the exposed individual has or develops symptoms, the person should be transferred to a medical facility with BSL-4 containment capabilities, until proper assessment of the individual is accomplished. If an exposure occurs and the individual does not have or develop symptoms, procedures for quarantine of the individual should be developed with specific guidelines as to the length of quarantine required if the person remains asymptomatic. If an individual becomes symptomatic and there is no evidence of an exposure, the individual should be treated as appropriate for the symptoms and monitoring should continue as prescribed by the Draft Protocol.

- *After Completion of Life Detection/Biohazard Testing:* The question of how long to continue monitoring has to be addressed. Certainly the duration of monitoring will be influenced heavily by the outcomes of the Life Detection and Biohazard Testing. Several factors may need to be considered in this decision, such as the protection of the workers versus the protection of the general population. Clearly articulated decisions will be needed on whether to have lifetime surveillance for the personnel or a mandatory period followed by optional reporting, if the risk was determined to be low. Certainly monitoring may become optional if the samples are deemed safe by the Life Detection and Biohazard Testing. Whether or not surveillance is needed for relatives or people living close to the workers should be considered. A distinction should be made between monitoring for risk management and continued collection of data for a research study. The interpretation of personnel evaluations may require the use of a control group or population-based estimations of frequencies of different events. If so, sources for this information should be defined.

Monitoring at Secondary Sites

The level of monitoring to be used at secondary sites receiving and working on portions of the Mars samples should be based on the results of the Life Detection and Biohazard testing. If the Mars samples are still potentially hazardous, several points should be considered in the development of a procedure for monitoring at secondary sites. First, secondary sites should be identified prior to the

31. Information on the Millennium Cohort can be found at http://millenniumcohort.org.

arrival of the Mars samples, to allow for pre-certification of personnel and collection of baseline data. Second, all distributions should be tracked and procedures for monitoring of containment at the secondary sites should be developed. Third, consider monitoring personnel at secondary sites using the same procedures as used at the SRF. The number of personnel at secondary sites is expected to be a small number of individuals.

If the Mars samples are deemed safe either through "sterilization" or by biohazard test results, then methods should be used for tracking all sample distributions and all individuals in contact with the samples. In this case, only event reporting is needed.

Database Issues

A central database facility with data analysis capabilities and procedures should be used to gather and maintain an (e.g., baseline, monitoring), personnel data (e.g., baseline, in-process, follow-up), secondary site data and sample tracking data. Procedures for regular data analysis and reporting should be developed. Access to and confidentiality of the data should be defined and assured. Data analysis should distinguish between surveillance and research, with consideration given to the need for ethical review and approval for research procedures.

Points of Consensus

- Personnel should be educated about procedures and risks for working in the SRF.
- Baseline and monitoring data should be collected using standardized tools.
- Surveillance of personnel and environment is an important component of the Draft Protocol.
- A central database facility should be available.
- Safety and surveillance issues should be included in public reporting.

Points of Discussion Without Consensus

- Should monitoring be restricted to relevant public health measures as opposed to extending the Draft Protocol to allow for epidemiological research?
- What time frame should be used for monitoring of personnel: lifetime versus limited period (according to hazards)?
- If long term monitoring is implemented, what parameters should be monitored on a long term basis?
- What level of medical facilities are needed at the SRF?
- What level of baseline and testing is required for secondary site workers versus primary site workers?

Summary

Monitoring procedures for personnel and the environment should be developed considering international regulatory, cultural, and ethical issues. Procedures for the monitoring of personnel should include procedures for education and certification. The radiation and chemical risks are considered of low probability and can be assessed early in the chemical testing procedures to reduce the monitoring burden. Develop procedures for database management and data analysis with assurances for confidentiality and security of the data.

Sub-Group 5: Requirements of Draft Protocol for Facilities and Equipment

Charter

The charter of Sub-group 5 was to examine the "Requirements of the Penultimate Draft Protocol for facilities and equipment: What? Where? When? What if [a life-form or biohazard is detected]? What are the advantages/disadvantages of distributing the protocol activities among more than one containment facility? What factors should be considered in sizing the primary containment facility? What requirements should be met by secondary (PPL-α, BSL-4) facilities? Are there any other considerations that should be taken into account in providing a facility the capability to enact the final protocol?"

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Size and Scope of the Facility: The size and scope of the facility will depend on the decision whether to conduct all final protocol tests at the primary site or to distribute the final protocol functions and activities to secondary labs outside the facility. A cursory review of the proposed tests and probable equipment requirements from the PWDP suggests that the facility must be expandable and flexible.

Where to Locate the Facility: The decision of where to locate the primary facility was not considered to be a scientific issue, excluding obvious cautions to avoid placement at sites subject to severe natural hazards (e.g., active faults, flood plains, etc.). The Sub-group reviewed the recent COMPLEX report on a Mars Facility *[SSB 2002]* and did not concur with their decision that this facility should necessarily be co-located with a BSL-4 facility. However, given the importance of maintaining this facility as an international resource for exploratory science studies, the Sub-group favored a location with ready access to an existing labor pool of scientists.

When to Begin Design of the Facility: Regarding the question of when to begin design of the primary facility, the Sub-group recommended as soon as the mission is certain and the funds have been allocated. Ideally, the design of the primary facility should begin now.

Distributing Protocol Activities Between Multiple Facilities: In discussing the question of whether or not to distribute final protocol activities and functions to more than one laboratory or facility, the Sub-group compiled the following lists of advantages and disadvantages:

Presumed Advantages:

- Corroborating scientific results
- Possible cost savings by use of existing facilities
- Simplifies design of initial facility
- Ability to have increased instruments
- Better participation of international partners

Presumed Disadvantages:

- Logistic and transportation difficulties
- Management difficulties (e.g., loss of control, uncertain cooperation)
- Potential danger of discovering biohazard after distribution
- The need for increased sample volume for testing
- More staff potentially exposed in the event of biohazard

What if a Life-Form/Biohazard is Detected? The facility must have the flexibility and be able to expand quickly to accommodate scientific research by principal investigators and researchers. Samples should not be released from containment for broader distribution.

What Requirements Should be Met by Secondary Facilities? Secondary Facilities must follow the same standard operating procedures (e.g., for staff monitoring). All work must meet the PPL containment guidelines based on testing needs. A "chain of custody" must be established for all samples transported between facilities. Security assessments must be performed.

The deliberations of Sub-group 5 were based on two primary assumptions: 1) Initial Life Detection is primarily for extant life (e.g., active or dormant) or biomaterials. The search for evidence of fossil life will likely intensify and continue after the samples are released from containment; and 2) Any extant life should be considered a biohazard.

The Sub-group failed to reach consensus on whether to endorse the notion of distributing protocol activities and functions to laboratories and facilities outside the primary facility. The majority opinion was to limit Protocol activities and functions to one major facility, perhaps with the exception of having a duplicate back-up holding facility for the banked Mars samples. This initial facility should have the ability to receive the samples and perform all tests at containment levels ranging from PPL-α through γ before distribution to any PPL-δ laboratories. However, the Sub-group agreed that there is nothing inherent in planetary protection requirements that would preclude the use of multiple facilities or sites to receive and process these samples. Therefore, the final decision about having a single or multiple facilities may depend on political considerations and finances. However, there was also a broad based consensus on two additional issues: 1) given the anticipated cost and unique design of the SRF, it would be advisable to build the facility for continuous operation to support other astrobiology research activities or those in biological or micro-circuitry sciences; and 2) the primary facility must be expandable and flexible.

The Sub-group also reviewed the Penultimate Working Draft Protocol and from it developed a schematic showing the sequential containment in which the samples will be processed prior to distribution to the broader scientific community (see Figure SG5-1).

Using the PWDP for Physical/Chemical tests, Life Detection analyses, and Biohazard assessments, the Sub-group compiled a rough inventory of the proposed tests and bench-top footprint of the requisite instruments. Based on this inventory, the Sub-group suggested that any individual PPL laboratory will not need to be large.

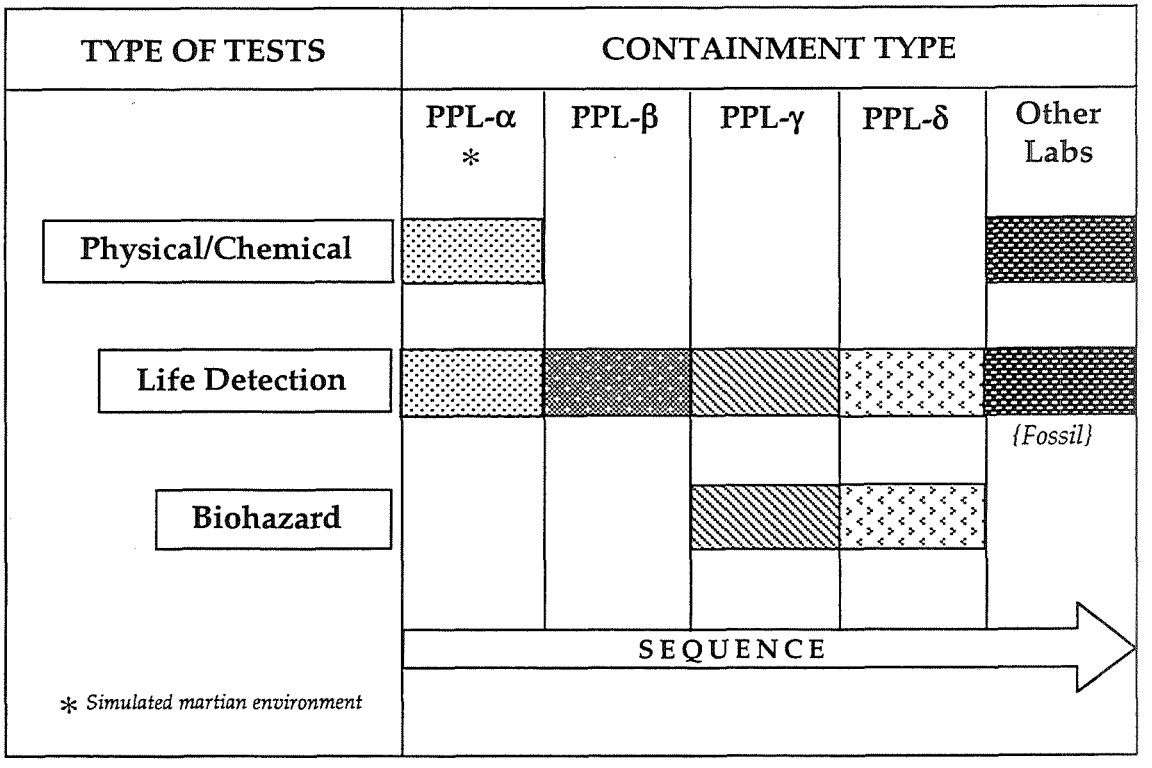

Figure SG5-1. Sequential containment requirements by test category
(see page 74 for the definitions of PPL-α, PPL-β, PPL-γ, and PPL-δ).

Recommendations on Additional Issues: Finally, additional issues identified by Sub-group 5 resulted in the following recommendations:

- Completely define the PPL containment guidelines.
- Develop schematics for a self-contained containment structure that could be placed in a BSL-4 laboratory and as a composite could meet PPL-α containment requirements. This structure should be able to use remote robotics to handle the specimens.
- Develop a comprehensive list of equipment required for all proposed tests.
- Anticipate the need to do some Life Detection tests under simulated martian environmental conditions while maintaining PPL-α/β containment.
- Put agreements in place with any anticipated PPL-δ labs prior to receipt of Mars samples.

Sub-Group 6: Contingency Planning for Different Draft Protocol Outcomes

Charter

Sub-group 6 examined the Penultimate Working Draft Protocol to consider contingency plans for various outcomes of the protocol: "Given the various possible outcomes of the different protocol elements, what should be done at/in/around the containment facility(ies) if: 1) Absolutely no evidence of organic material is found in the sample? 2) The results from the protocol (especially Life Detection/Biohazard testing) are contradictory/inconsistent? 3) A self-replicating entity or biomaterial(s) indicative of extant life is discovered within the sample materials? and, 4) That self-replicating entity cannot be shown to represent a terrestrial contamination."

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Sub-group 6 was asked to anticipate how the scientific community would react under a variety of possible scenarios following the return and testing of martian samples. In addition to considering the scenarios in the original charter, the Sub-group also included the question of how to respond in the face of possible breaches in containment.

'Lessons Learned' From Workshop 3

Sub-group 6 began its discussions by considering information from the plenary discussion of Workshop 3 and identifying several points relevant to their charge.[32] In particular, analogies with the public reaction to the announcement of possible evidence of life in meteorite ALH-84001 should be anticipated. Press coverage is likely to be intense, reflecting public interest. An oversight committee may be necessary to handle the public dissemination of information in an orderly way and this may represent an opportunity for public education about how science works.

32. "What if Life is Detected" *[Race et al., 2001b, pg. 41].*

The Sub-group identified a number of other issues needing additional discussion. If evidence for any life exists in the samples, then containment for an indefinite time should be considered. There may then be a need for changes in the anticipated procedures as well as the equipment and facilities required. Ethical, legal, and social issues should be considered seriously, and expertise in these areas should be reflected in the membership on the oversight committee. Other important issues will include concerns about security, especially from potential disruptive activities of any "radical" groups that may be opposed to sample return. Although it is difficult to anticipate the kinds of practical applications that might result from discovery of new forms of life, it will be important to protect the public's rights to any intellectual property potentially associated with extraterrestrial samples.

Findings and Recommendations

After considering the specific scenarios and questions in the charter, Sub-group 6 made the following findings and recommendations, presented here in a reverse order from how they were listed in the charter:

Breach of Containment: The responses to a breach will depend on where it occurs and what happens. Conceivably, it could occur in an area with a high population density or in a remote location. The breach could be a result of an accident or a crime – as a result of activity either outside or within containment. The consensus of the Sub-group was that we know basically how to handle breaches based on long term experience and emergency plans for handling pathogenic biological material under BSL-3 and BSL-4 containment. Additional information for responding to breaches and containment problems has been gained through decades of experience in handling lunar and extraterrestrial materials.

Clearly, an emergency plan will be needed well in advance to develop recommended responses to various breach scenarios. The first steps would involve investigation of the degree of compromise, considering both biosafety and sample integrity. Full documentation of any breach event will be required as well as identifying the degree of sample compromise, what organizations or personnel should be involved in all phases of a response, and how notifications and communications should be handled. The plan should focus on all aspects of mitigation, cleanup, and recovery from perspectives of both biosafety and sample integrity (e.g., decontamination of the area; sample recovery, re-packaging and labeling as compromised, or destruction if required, etc.)

Containment Facilities: Based again on experience with handling of pathogenic biological material, multiple locations for facility functions may be beneficial. This would add a redundancy, and also increase the security of the samples by distributing the collection in case of loss at a single site (e.g., from natural disasters, accidents, or illegal acts). The biohazard, curation and security equipment is well known, and should be based on current biomedical and counter-bioterrorism efforts.

Facility Management: A Facility Administrator should be present on site to make day to day decisions about facility management, acting under final protocol guidelines established by an oversight committee. The committee should be on call as needed by the Facility Administrator, especially for non-anticipated scenarios. Every effort should be made to coordinate the administration with relevant government agencies.

Non-Terrestrial Life Confirmed: In keeping with the SSB recommendations *[SSB 1997],* sample materials will be released from containment only if they are shown to contain no extraterrestrial life-forms, or they are sterilized prior to release. If a portion of a sample is confirmed as positive for non-terrestrial life, subsequent testing and analyses on all samples materials will continue in containment. This means that all physical, chemical, and geological characterization, as well as Life Detection and Biohazard tests requiring un-sterilized material should continue to be done in strict containment, either at the SRF or any other test facilities that may be used. Experimentation on conditions to sterilize the newly discovered life should begin immediately. Once sterilization procedures can be confirmed and re-validated, detailed plans for distribution of samples should be developed or revised based on the latest findings. Management issues will include administrative and technical procedures for scientific study and curation, as well as informing the public.

Extant Life or Biomaterial Positive: If extant life or evidence of biomaterials are detected in the samples, all work on the samples will be done in containment facilities. Maximum effort should be made to determine if the positive results are originating from Earth life or Mars life. Information management becomes an issue both for scientific communication and debate among scientists as well as how initial information, with its attendant uncertainties, is disseminated to the public.

Organic Carbon: It is extremely likely that carbon will be found in sample materials. The sensitivity of current and future methods will be very high, so that at least some degree of contaminants will be detected. The existing base of knowledge on meteorites and other material collected from space will be useful in providing baseline information to help guide the investigations. Since the Viking results focused on volatile organics, *in situ* measurements of non-volatile organics would be useful to predictions of anticipated sample organic content.

Contradictory/Inconsistent Results: Given the number of techniques, spanning several scientific disciplines, it is very likely that contradictory or inconsistent results will be found. Differences in the sensitivity of methods will exist and confidence in the level of controls will differ. It will be important to stress replication of experiments and duplication of results among multiple sites to add confidence to the results assessed.

No Organic Material Detected: There was disagreement among the Sub-group members as to how to handle samples that were devoid of organic material and had no evidence of life. According to the COMPLEX Committee, *[SSB 2002]*: "If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, release of un-sterilized aliquots of the samples for study beyond the confines of the Quarantine Facility is justified."

Despite the recommendations from COMPLEX, some members of the Sub-group felt that an increased assurance should be given prior to any sample distribution and that some sterilization procedure would be advised. The method of 'prophylactic sterilization' proposed would involve gamma radiation and minimal heating. An opposing view was that the scientific method inherent to the Draft Protocol is designed to test for significant biohazards and thus, the Draft Protocol should be followed. Blind changes to the Draft Protocol would destroy some significant scientific value of the sample while unnecessarily eroding public trust, and adding nothing to the assurance of public safety.

Because Sub-group 6 was unable to reach consensus on the question of 'prophylactic sterilization,' their arguments and counter arguments are presented in the accompanying Text Boxes 1 and 2.

Sub-Group 6, Text Box 1: Argument In Favor of 'Prophylactic' Sterilization of Mars Samples

The rationale for 'prophylactic' sterilization of Mars samples prior to distribution for geochemical studies is based on the following facts and logic:

- First, the samples that have been subjected to life and biohazard detection analysis cannot be sent out to the geologists because they have been consumed or saved for further long-term or verification studies. The samples that will be sent out, will not have been subjected to this analysis. The detection analyses cannot be assumed to represent all of the material, because it is not homogeneous. Every pebble and rock has anomalies which are unique, and represent likely sites to look for unusual geochemistry (and for life). Even sand grains, although randomized by mixing, are unique, one from another, and are laboriously sorted by hand for Earth studies.

- Second, a dozen or so cell systems and organisms will be tested for biohazard, out of about a million extant terrestrial species. Ecosystem interactions cannot be tested in any feasible manner in a high containment lab, nor can the possibility be excluded that some Earth agent (e.g., virus, viroid, prion, plasmid) might establish an interaction with a Mars organism to produce an otherwise absent danger (e.g., bacteriophage). Thus, while the finding of life or biohazard gives a new and dramatic area for further study, negative results cannot assure either the safety of the remaining material, or the absence of danger to the vast majority of non-studied earth species and the possible consequent effects on the ecosystem.

Now, we recognize that these possibilities are very unlikely, but if they are not credible at all, then why are we mandated to build an exquisite containment facility for the initial studies? If we are obliged to consider these dangers as credible, then why not provide an increased level of security by employing a sterilizing level of irradiation, especially if it is virtually harmless to geochemical characteristics? Published studies indicate that 30 Megarads, an enormous dose for biological sterilization, is virtually harmless for geologic studies. Further experiments might push this to 100 Megarads. This would satisfy all but the most extreme extremists among biologists.

Nor must we plead ignorance in dealing with the possibility of non-carbon based life. Unless we believe in disembodied spirits (hopefully rare among scientists), we can place obvious constraints on possible life-forms regardless of their atomic basis. The chemical elements on Mars are the same as those on Earth, and the strength of interatomic bonds are all quite similar. In order to defeat entropy, life must contain polymers to provide specific information and reactions, and must be separated from the environment by a membrane (viroids and prions are parasitic, and could not have originated life, which must begin with an autotrophy). Irradiation breaks bonds, whether carbon-based or other element-based, yielding monomers to small oligomers, which cannot provide the information, specificity, or isolation essential for life. Thus, the abundant data on sterilization of terrestrial items by destruction of microbes is directly applicable to martian 'life-as-we-don't-know-it.'

Finally, it seems unreasonable (and unscientific) to fear 'sterilizing' samples because it would arouse public doubts. Surely the public, as well as scientists, can appreciate the benefit of an extra margin of safety.

Sub-Group 6, Text Box 2:
The Hazards of 'Prophylactic' Sterilization of Mars Samples

Sample lots that have been subjected to life and biohazard detection analysis *can* be sent out to the geologists and to other scientists (e.g., exobiologists, etc.) after they are tested, if representative sub-samples can be allocated to each purpose. Whether this can or cannot be done for a particular sample portion is entirely dependent on the nature of that portion. Real-time judgment will have to be applied to the decision on whether the release of a sample can be made based on the tests on a representative sub-sampling.

- Life-detection protocol testing is expected to detect organic materials at the femtomole level, both destructively and non-destructively. This screening will be combined with elaborate biohazard challenge testing—which is not designed to stand alone, but as a complement to life-detection testing. Viruses, viroids, plasmids and prions all need to have organic material (even living organic material) to replicate. Organic material associated with these Earth-entities will be a special target of the life-detection protocol.

- It is simply not the case that heating above 80°C or the application of 30 Megarads of radiation are harmless to geological studies. What may not affect geochemistry can be devastating to mineralogical or petrographic studies of a sample material—or the detection and interpretation of potential biochemical evidence of past life. These processes may not provide any additional "security" in the release of a sample for outside study—especially if there are no credible risks remaining after protocol testing—but they may have serious negative effects on sample science.

- A goal of the protocol development should be to develop a process whereby rational testing for known or suspected biomaterials and biohazards can be accomplished—even if those hazards eminate from "life as we don't know it." A sterilizing process that is thought to be effective against envisioned carbon-based or non-carbon-based life is useful in certain phases of such a Draft Protocol to allow for specialized sensitive analyses outside of containment—but such a process is not a magic wand. Heat and/or radiation break bonds to be effective, and as such they destroy evidence that may otherwise lead to the very discoveries that the Mars sample return mission is being designed to seek.

Resolving these arguments about prophylactic sterilization will be essential to an evaluation of a Mars sample handling protocol – and indeed ultimately to effectiveness of a Biohazard testing altogether. Central to the arguments for and against sterilization, however, is the question of risk – can *any* protocol be guaranteed to be absolutely risk-free?

If not, what is an acceptable level of risk (for example, one that approximates the risk from the natural influx of martian materials into the Earth's biosphere)? And is there any treatment method that can eliminate all risks from the returned samples while preserving them for the detailed scientific study envisioned by scientific community? These questions were debated in the plenary session during the report of this Sub-group. Clearly, the issue of sterilization will require serious additional attention and research well in advance of sample return.

Sub-Group 7: Personnel Management Considerations in Implementing the Draft Protocol

Charter

Examine personnel management considerations for implementation of the protocol: What are the requirements for personnel to complete the Penultimate Working Draft Protocol, as written? When do personnel need to be hired and trained? What considerations can be given to the qualifications of required personnel, and the selection process by which personnel are chosen to: 1) conduct the various elements of the Draft Protocol? 2) provide for the appropriate biosafety considerations and containment at the primary and any secondary facilities? and 3) conduct any required analyses that are of scientific interest or are also necessary to support preservation and curation of the martian samples (e.g., time, processing-dependent studies)? What external advice/oversight capabilities should be available to support the execution of the sample handling protocol (e.g., to ensure that the Draft Protocol is executed according to plan, and that if modifications are necessary they are approved and documented)?

During the course of the general sessions of Workshop 4, the Sub-groups were also requested to address personnel and communication management considerations associated with the design and construction of the facility(-ies) and the implementation of the final protocol.

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The Sub-group had a thorough discussion about the alternative methods for staffing the facility(-ies), either through the recruitment of scientists in permanent positions or through the presence of working scientists on-site at the facilities, selected though a competitive grants process. The conclusion was reached that various categories of personnel will be required depending on the different tasks that need to be implemented. The management of the Mars Sample Return (MSR) program will then propose the different origins of the personnel. The Sub-group agreed to the following:

- Personnel should be hired progressively during the development of the project and the facility(-ies). After considering the many functions that will be needed for implementation of tasks during the design, building and operation of the facility(-ies), the Sub-group suggested that the functions and responsibilities of the director's position may be carried out by appropriate committees until a Director is hired which should not be later than about five years before the return of samples from Mars.
- The required methods and procedures outlined in the Protocol should be applied to any facility or site handling martian samples during the implementation of the Final Protocol;
- The international character of the program should be respected throughout the whole process.

The Sub-group developed its suggestions on the design and construction of a dedicated Mars Sample Receiving Facility (SRF) without precluding the possibility that some activities during the containment could be performed in other existing facilities, perhaps remote from the SRF. They interpreted the outcomes of previous workshops as leaning towards the design and the construction of a dedicated facility(-ies) to handle the samples and to perform some (most) of the tasks of containment. In their deliberations, Sub-group 7 developed an overview of the functions, staffing requirements, and organization that will be needed to design, build and operate a Mars SRF. Figure SG7-1 shows a high level schedule and overview of the process leading up to sample receipt.

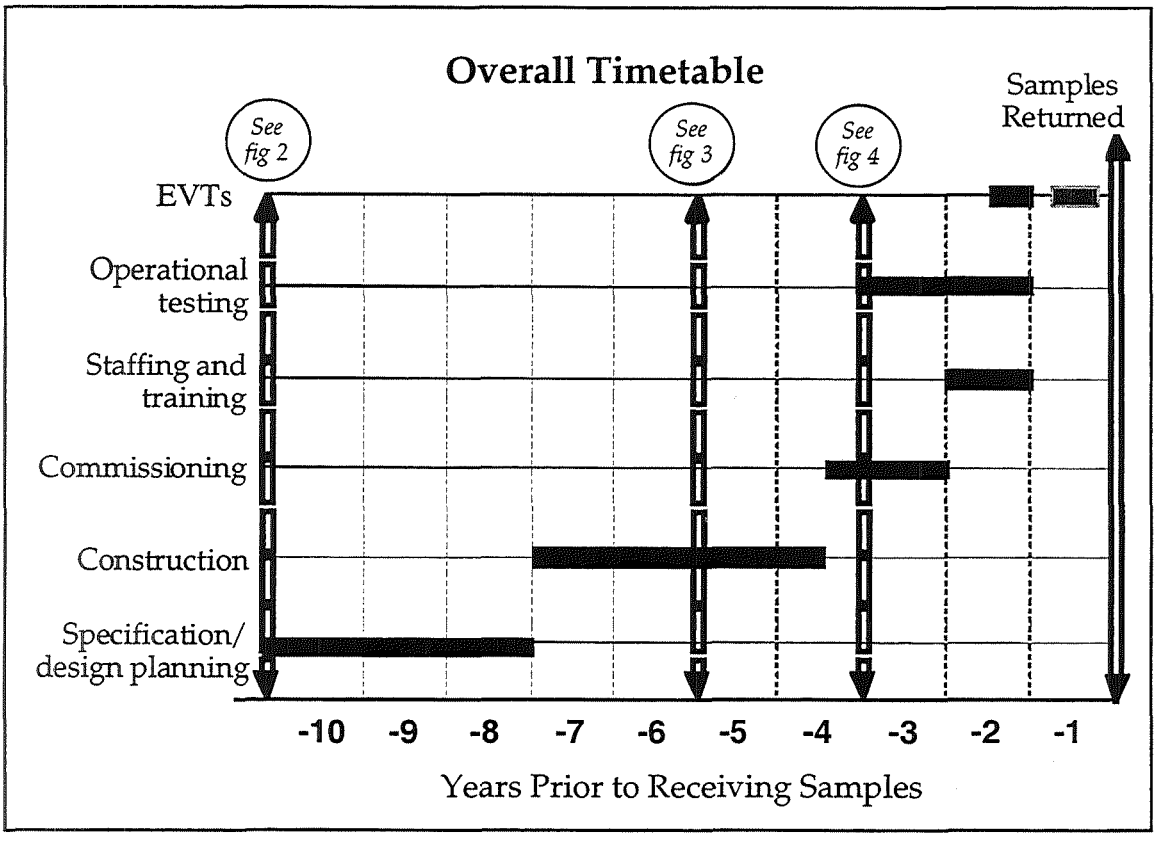

Figure SG7-1. Possible overall timetable of the activities required to design, build, and operate the SRF. The double-headed arrows indicate the time of the possible staff organization described in the subsequent figures. (EVTs = Experiment Verification Tests).

In developing their suggestions for management and staffing of the SRF, the Sub-group used the following assumptions behind their working hypotheses:

- The Protocol must be fully and successfully tested before actual handling of martian samples.
- It is estimated that a complete series of Experiment Verification Tests (EVTs) will last approximately 6 months and one complete series of EVTs must be successfully demonstrated before actual handling of the returned samples. The first series must begin no later than 18 months before the returned Mars samples arrive in order to allow enough time to adjust and repeat the series if necessary (at least 9-10 months before experiments begin on actual returned samples).

- The EVTs are consistent with the recommendation of the SSB (1997) and earlier workshops in this series that the SRF be operational two years before the arrival of the actual Mars samples. The EVTs are part of the normal operational testing.
- Based on experiences at other BSL-4 laboratories in the United States and France, no less than one-year is required to properly staff and train the technical and scientific personnel.
- Commissioning of the SRF, which must be performed in parallel with the staffing and training, will last at least 18 months.
- In order to accommodate the staffing, training and commissioning requirements of the SRF, construction of the facility must be finished 3 years before the actual operations. From past experiences in both France and the United States, construction of the facility itself will also require 3 years.
- It is estimated that about 3 years will be needed to develop design specifications and plans for the SRF and obtain necessary authorizations for the facility. To accommodate all the activities necessary to design, build and operate an SRF, the entire process must begin fully ten years in advance of sample return.

Figures SG7-2 – SG7-4 provide details on possible organizational and staffing levels at three key times identified in Figure SG7-1: 10 years, 5 years, and 3 years prior to sample return. Specific details related to the possible staffing and organizational plans are provided below; exact positions, job descriptions, and expertise requirements for various positions are all TBD.

As soon as the decision is made to build and/or update a Mars sample receiving facility, typically 10 years before the actual operations, four positions must be filled in order to prepare the specifications and review the design of the facility (see Figure SG7-2): the Director, Deputy Director of Administration, Deputy Director of Science, and an Environment, Health and Safety Officer.

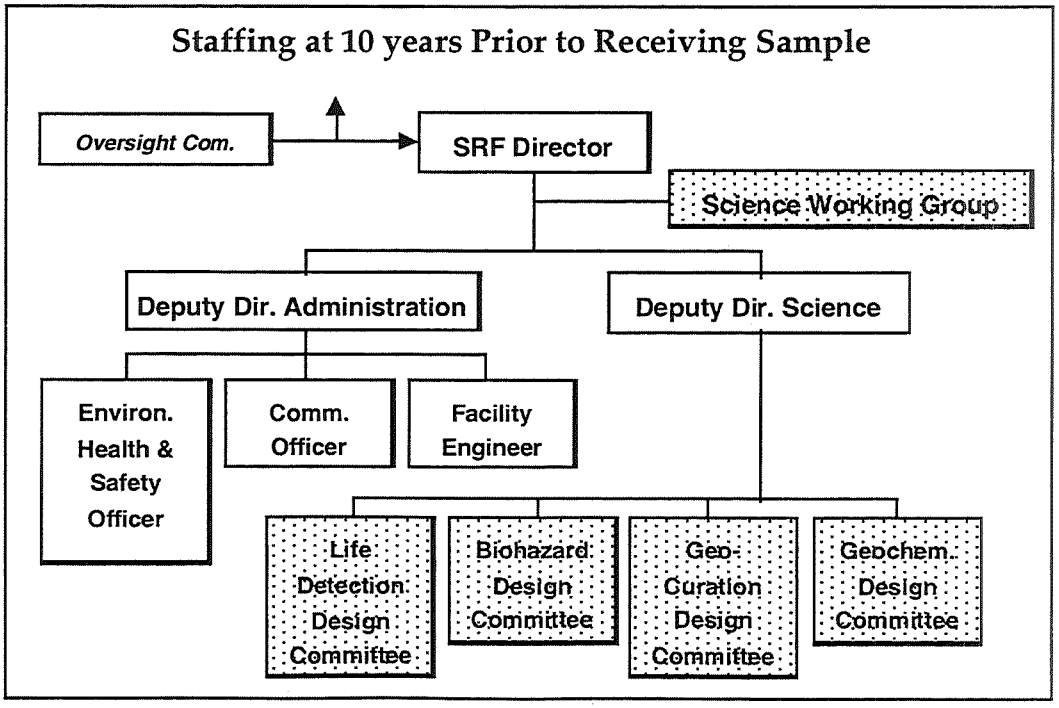

Figure SG7-2. Staffing requirements and structure of the SRF at 10 years prior to arrival of the returned sample(s) (permanent positions are in plain boxes; committees are in stippled boxes).

The Director will work under the scrutiny of an Oversight Committee that will check the compliance of the project development with the Final Protocol. The Deputy Director of Administration will be assisted by the Environment, Health and Safety Officer who will deal with the actual design requirements related to these critical topics. In addition, the Communication Officer will be in charge of risk communication and outreach, keeping the community informed and answering questions regarding the SRF. A Deputy Director in charge of Science will coordinate the work of four scientific committees that will develop specifications and follow the design process for their respective disciplines or areas. A Facility Engineer will work with appropriate design committees to coordinate planning, design, and building of the SRF.

At roughly midway through the construction of the facility, the Sub-group recommends hiring the Heads of Staff (H of S) for each scientific discipline required (see Figure SG7-3). These people will ensure that construction is properly completed to accommodate the specific needs of their disciplines. With the help of their respective advisory committees they will prepare the general and specific operating procedures to handle the martian samples and the training program for staff to be hired. At this point, an Administrative Manager will also be hired to organize the actual staff and prepare for future administrative and personnel needs.

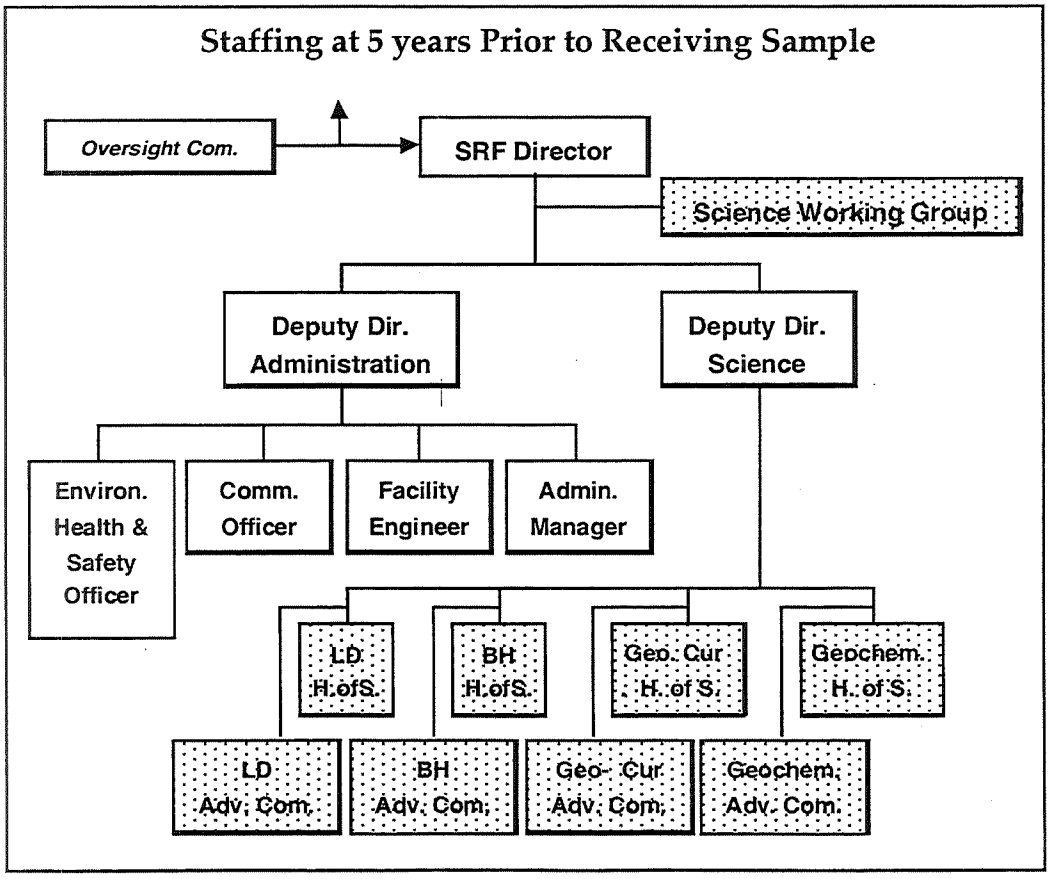

Figure SG7-3. Staffing requirements and structure of the SRF at 5 years prior to arrival of the returned sample(s) ("H of S" = Head of Staff; permanent positions are in plain boxes; committees are in stippled boxes).

In order to have a fully operational facility two years before samples are returned, the final staffing and training of various operational positions must begin three years prior to actual operations (see Figure SG7-4). At this time the Institutional Bio-Safety Committee (IBSC) and the Institutional Animal Care and Use Committee (IACUC) will be installed.

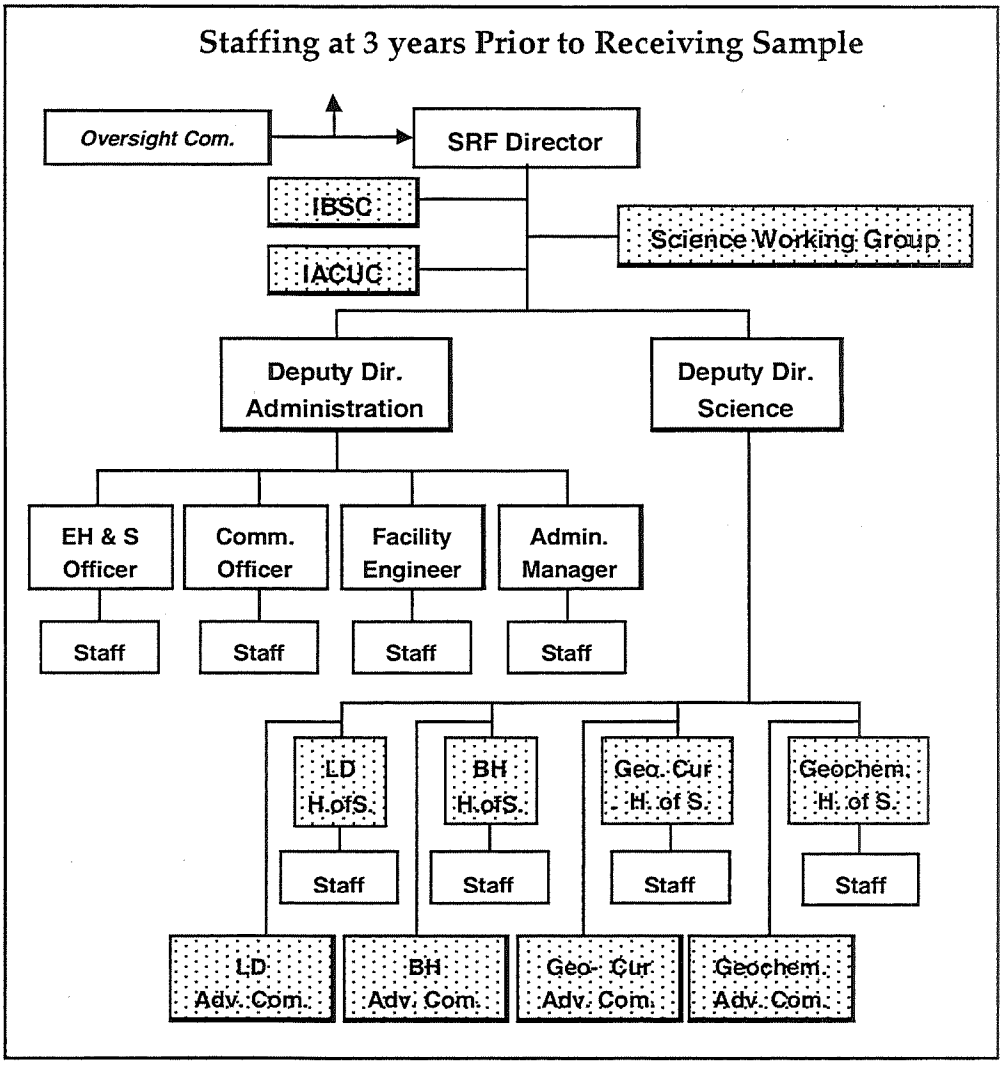

Figure SG7-4. Staffing requirements and structure of the SRF at 3 years prior to arrival of the returned sample(s) ("H of S" = Head of Staff; permanent positions are in plain boxes; external committees are in grey boxes; committees of staff and external members are in stippled boxes).

From the beginning of the process, the Sub-group recommends that three different of committees be installed to help the Directors and Heads of Staff in overseeing their changing responsibilities:

- Scientific design committees will be specialized in the four disciplines (Life Detection, Biohazard testing, Geo-curation and Geochemistry). The members, who will be prominent scientists, will be designated by the agencies. These committees will prepare the design, review the project and oversee the project to ensure the facility can operate consistent with the operational aspects of the Protocol. As soon as the Heads of Staff are hired, these committees will shift to become Discipline Advisory Committee helping the Heads of Staff.

- The Science Working Group will be in charge of reviewing the project and the construction to ensure its compliance with the scientific requirements and the Final Protocol; they report and comment to the SRF director in coordination with the Deputy Director of Science.
- Finally the Oversight Committee is to be composed of 12 to 15 members selected through the same method as the members of the NASA Planetary Protection Advisory Committee or the French Planetary Protection Committee. This committee will be charged with reviewing the overall process and the proposed measures to comply with the requirements of the Final Protocol. The committee will report to the Director of the SRF and above.

It was recommended that membership on the various committees be staggered to insure an appropriate turnover without losing the memory of the project (e.g., two people rotate off every year). In addition, the Sub-group recommended that the agencies set up an international search committee for recruitment of the Directors, functional officers, the Facility Engineer and the Heads of Staff.

Finally, the Sub-group identified three major issues for further consideration:

- Currently, no one has experience in simultaneous operations or activities in BSL-4 and clean room conditions as will be needed for maintaining PPL-α through PPL-γ. The advice of experts from the pharmaceutical or the microprocessor industries would be helpful.
- Details on the optimal staffing mix at the SRF must be considered further. It is not clear what mix of civil servants, semi-permanent employees, contractors, and guest scientists will be needed to staff the facility and implement the Final Protocol. However, international access and participation should be considered throughout planning for staffing and operations.
- In order to comply with planetary protection constraints and final protocol requirements, a sustained and adequate budget will be needed throughout the design, building and implementation phases of this project.

Sub-Group 8: Draft Protocol Implementation Process and Update Concepts

Charter

Sub-group 8 addressed issues related to the processes to implement and update the Penultimate Working Draft Protocol: "How should the final review and modification of the protocol be conducted? What steps should be taken in gaining approval of the final protocol by national and international bodies important to its acceptance/implementation? How should the protocol be maintained after its initial approval and promulgation? What steps should be available to the protocol implementers to provide for proposed changes in the details and/or framework of the protocol once it has received initial approval? What process should be followed to reaffirm acceptance/approval of the final protocol to be used for the actual samples? What regulatory steps (if any) should be taken to certify the samples are safe for release from containment after the protocol is completed?"

Sub-Group 8 Members

Bielitzki, Joseph (U.S. Co-Chairperson)
Cambon-Thomsen, Anne (French Co-Chairperson)
DeVincenzi, Donald L.
Fultz, Patricia
Hoyt, Diana
Race, Margaret
Rummel, John
Sogin, Mitchell
Treiman, Alan

The scope of the assigned task as seen by Sub-group 8, is summarized in Figure SG8-1. A narrative explanation of recommendations and activities at each stage is provided in the text that follows. Several fundamental issues were raised prior to the discussion of the specific charge to the Sub-group. Because of their importance to the overall Protocol, they are also detailed as follows:

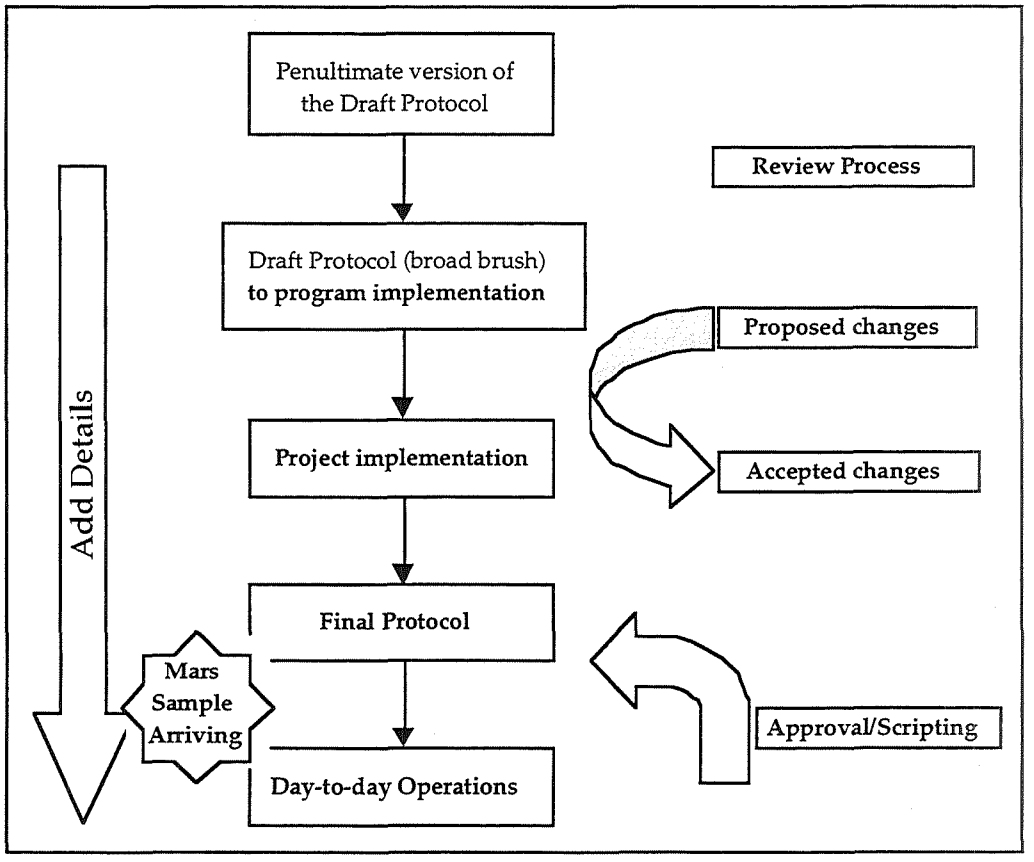

Figure SG8-1. Protocol Implementation and Up-date Process.

Clarity of Meaning and Terminology: Sub-group 8 agrees with several other Sub-groups that clarity of meaning is essential to the implementation of any process especially when the process involves international agreements. Therefore, it is again recommended that absolute consistency be used in the language for these documents and that when the actual definition of a word or phrase is in dispute, reference should be made to those definitions or meanings that are standard and accepted when interpreted at the international level.

Use of Planetary Protection Levels (PPL) rather than Biosafety Levels (BSL): A second area of concern is the use of "Planetary Protection Level (PPL)" synonymously with "Biosafety Level (BSL)." The PPL designation is intended to define a safety system consistent with the needs of the Mars Sample Handling and Return Mission that take into account pristine isolation of the samples, personnel safety and environmental safety. The BSL system varies in definition between the United States and Europe, and varies even within the U.S. between the CDC definitions and requirements,

(which focus on protection of people), and the agricultural definitions (which have less emphasis on people and greater emphasis on the environment). Sub-group 8, therefore, recommends that a uniform system of nomenclature and definition be used throughout the process that stipulate only the PPL designations. This will prevent confusion and result in more uniform application of safety standards and practices.

Planetary Protection Level Delta: In addition to noting the discrepancies in BSL terminology above, the Sub-group also identified the need for naming a new level of containment, PPL-δ. This newly defined and named containment level would apply to situations at the point in the protocol where samples do not require atmospheric isolation and may be moved to external laboratories with proper facilities for further analysis. Sub-group 8 recommends this additional PPL definition, Planetary Protection Level 'delta,' as:

> *"[PPL-δ] Provides a level of containment for the samples that allows investigators to work with samples in a laboratory situation, which provides protection to personnel through an engineered environment providing HEPA filtered air entering and leaving the area, containment of water and/or waste to the laboratory and protection to personnel through personnel protective equipment consistent with the most protective standards referring to the 'U.S. BSL-3 Agriculture' and 'French P4' requirements."*

Final Scientific and Policy Reviews: Sub-group 8 recommends that the final review of the Protocol be subjected to the highest degree of scientific scrutiny and evaluation. The evaluation should be conducted jointly by scientific organizations from both the United States and France to avoid prolonged negotiations and resolutions that may arise when such reviews are conducted separately. This review should probably occur at the level of the National Research Council in the United States, and its equivalent scientific organization in France. The French participants agreed to investigate which of the French institutions is most appropriate (among the French institutions discussed were CNRS or representatives of various EPST, Etablissements Publics à Caractère Scientifique et Technique [including but not exclusively CNRS] or Académie des Sciences). Final decisions about which institutions should be involved in scientific reviews is TBD.

Ethical and Public Reviews: Evaluations of the proposal should be conducted both internal and external to NASA and CNES and the space research communities in the nations participating in the mission. An ethical review should be conducted at least at the level of the Agencies participating and these reviews made public early in the process (in France, the National Consultative Bioethics Committee, CCNE, is the appropriate independent organization). The Final Protocol should be announced broadly to the scientific community with a request for comments and input from scientific societies and other interested organizations. Broad acceptance at both public and scientific levels is essential to the overall success of this research effort.

Future Modifications to the Draft Protocol: When a Final Protocol has been adopted and approved by a consensus of appropriate scientific organizations, few changes should be made to its content. Changes should be made as scientific information, methodology and/or technology improve between the time of the approval and the actual physical implementation of the Final Protocol within the laboratory. The Sub-group recommended that changes in the Final Protocol methodology may be considered if a proposed change would meet the following criteria:

- increases the sensitivity or selectivity of the test,
- reduces the length of time necessary for a test without a reduction in sensitivity or selectivity,
- reduces the complexity of the sample handling process,
- increases the overall safety of the process,
- reduces the chances of contamination to the sample or the environment,
- reduces the cost of the process, or
- represents a new technology or method that has the broad general acceptance of the scientific community.

Advisory Committees and Expert Panels: Changes to scientific methodology and instrumentation are inevitable due to the long development time envisioned for this mission. This necessitates long term, consistent input and advice from the external scientific communities of all the partners engaged in the process. To facilitate this process, it is recommended that a standing Planetary Protection Advisory Committee (PPAC) be appointed in the U.S. to provide input to the Planetary Protection Officer and that a similar standing committee (Planetary Protection Committee, PPC) be appointed in France as tentatively planned.

The Sub-group also recommends that standing joint (French and U.S.) working committees or specialized expert panels should be appointed with appropriate expertise to provide support and advice to the U.S. PPAC and French PPC in each of three specific areas: technical processes, scientific procedures, and safety/biosafety issues. To provide the highest level of support to the process, these groups should be individual panels comprised of members with expertise in a particular area of concern. Individual experts should be limited to a single panel. The overall membership of the committees and expert panels should meet the specific needs of the Agencies and should represent the scientific goals of the Agencies and the external science communities. Their work should aim at providing the respective agencies with information essential to the success and safety of the Mars sample return missions. These panels and committees may function jointly or independently depending on the specific need.

The PPAC and French PPC will receive the annual reports of the three panels, who will also provide annual written reviews to the Planetary Protection Officer and, in France, to the appropriate Minister to which the committee reports. These reviews should include relevant operational issues and concerns and provide risk assessments of the technical processes, scientific procedures, and safety/biosafety plans and processes. These reviews should be made available to scientific and professional organizations with interests in the mission activities.

Communications: Unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels. Therefore a communication plan must be developed as early as possible to minimize the dissemination of misinformation and to provide the highest level of public assurance about the issues addressed by the mission. Communications should clearly inform about the extensive efforts to protect the environment, health and safety through facility designs, procedures, and personnel training. This information on risk management and planetary protection should be balanced with education/outreach about the anticipated benefits of Mars exploration and sample return from the scientific perspective. The communication plan needs to address the concerns of both the scientific community and those external stakeholders who will raise valid concerns on behalf of the world's population. In order to minimize long-term criticism and concerns, it will be

important to inform the public openly and honestly about all aspects of the mission in a way that provides accurate, timely details about scientific benefits, expectations, risks, and uncertainties. In particular, both the public and scientific community should be informed of results during Life Detection and Biohazard testing at appropriate times in the process based on procedures and criteria (e.g., level of certainty, consensus or majority, etc.) for determining how observations and data will be designated as results suitable for formal announcement. Details about who will be in charge of this communication plan and the release of information are TBD.

Flow Charts and Timelines: In order to assure the rational utilization of both the facilities and sample materials, appropriate flow charts and time lines must be developed to coordinate the complex series of interrelated procedures. Safety issues must be prominent at all significant decision points in the process (e.g., release from containment, downgrading to lower level of containment); these critical points must be identified and agreed upon in advance. Such flow diagrams are intended to coordinate complex testing and inclusion of all required elements, especially those concerning biosafety and biohazards leading to the sharing of sample material with the external scientific communities. Such flow charts, and time lines, should also include key decision points for changing the status of the sample to a less restrictive PPL and for proceeding in a particular direction along branches of the decision tree. Each such chart should contain an incorporated risk tree and assessment process.

Workshops/Reviews: The need to change schedules and procedures may be anticipated during the time between now and sample return. To provide assurance that rules exist between the involved international partners and the scientific communities, two workshops/reviews should be scheduled prior to sample return to Earth in order to reaffirm details about process, methodology, safety and release criteria. The first review should be conducted at the conclusion of the facilities design phase to determine if the physical structure meets the scientific and safety standards as defined within the specifications. In addition, the first workshop should review the existing procedures that will be conducted within the facility to confirm the specific flow chart outlining the approved sequence of tests and analyses. A second similar workshop/review should occur after the samples have been collected on Mars but in advance of their actual return to Earth for evaluation. Details about who should coordinate these workshop/reviews and modify schedules or procedures are TBD.

Preparations and Processes for Decision Making about Release of Samples: It will be important to prepare in advance for data interpretation and decision making in an organized way. This will be especially critical in the event that a distinctly martian life-form is found within returned samples. While it is impossible to develop details of the Final Protocol at this time, it will be crucial to have considered how decisions will be made, by whom, and based on what principles if an extraterrestrial life-form is discovered. A specific committee should be established at least a year ahead of sample return to develop contingency protocols and processes that will be in place if and when martian life is found and verified.

It is likely that protocol test results will not lead to unanimous decisions in all instances. It will thus be important to have a review and approval infrastructure for handling decisions about whether or not to release sample materials from containment, or reduce containment to a lower level, upon completion of tests. Addressing the overall decision making process in a formal manner will be critical for drawing conclusions, certifying results, and deciding whether samples qualify for release or not. Any decision to release samples should involve selected multidisciplinary experts and groups, as well as an

Interagency Committee on Back Contamination (ICBC), similar to that used in the Apollo program. The U.S. PPAC and French PPC should be involved in reporting to relevant bodies in their respective countries. Details on the organizational structure(s) associated with decision making are TBD.

The organizational structures, management plans, charters and reporting lines for many of the proposed committees and groups will need to be developed in the coming years. Many questions cannot be resolved until additional details on facility design, operational logistics, mission architecture or anticipated schedules are made available.

PLENARY DISCUSSION

What Areas of Research and Development are Needed for Protocol Implementation?

During a one-hour session on the final day of the Workshop, the participants explored the areas of research and development that would facilitate and/or enhance the implementation of the analytical aspects of the Draft Protocol. A number of areas were identified; they currently exist in varying degrees of development and are presented here in a brief outline-style (no attempt was made to specify details or parameters).

1. Improved Controls
   - On samples and tests – need both positive and negative
   - On personnel – requirements on clothing, operating procedures, handling of contingencies, etc.
   - Staff health and safety monitoring
2. Equipment for using Synchrotron Tomography
   - Refine and improve – bench top equipment (for both inside and outside of SRF)
3. Ecological Microcosms
4. Post-Radiation Detection of Biosignatures
   - How to make sense of organic molecules etc. after high doses of radiation
5. Further Discussion/Descriptions of Endolithic Communities
   - Taxonomy of rocks and where life is found in them (where to look; cracks, pores, etc.)
6. Microbiological Community Cultures
   - Characterization of cultures; un-culturable
   - How to find un-culturable microbes
   - Mixed Culture research
   - Containment Implementation
   - Micro-arrays for detection of microbes in rock
7. Robotics
   - Remote manipulation for use in BSL-4/clean room conditions (no experience)
   - How to disinfect suits, tools, equipment etc. in ways that don't mess up cleanliness materials
   - Procedures; manipulations; high through-put screening
   - Robotics to help maintain cleanliness and biocontainment
8. Transport and remote containment methodology/requirements
   - Sample containers for transport – IF materials transported beyond SRF holders for samples to go from instrument to instrument
   - Resolve problems of lubricants, etc.; other potential contaminants
   - Feasibility of a transportable PPL lab
   - Need to consider both biocontainment and cleanliness
   - Need to link containment & transport mechanisms with SRF design architecture
9. Organic Detection on Surfaces
   - "Remote" and non-destructive
   - Improve detection limits and resolution on both samples and lab surfaces
10. Miniaturization – nanoscale
    - Especially for testing and assaying techniques as part of the Protocol
11. Social Science Research
    - Psychology of working in maximal containment, highly restrictive environment
    - Operator short cuts; risk taking etc.
    - Sociological research on how communities respond to proposals for high containment labs

12. Sample Preparation and Handling
    - Tracking locations within samples
    - Sample register techniques – repeatability
    - Correlation with other sample features
13. Communications
    - When to release data; announcement processes
    - Criteria for determining quality data (interpretation)
    - Procedures for updating of raw data/when does data become 'findings'? revised findings?
    - Risks of announcing too early; too late? etc.
    - Rewards to individual – who gets credit?
14. Sterilization of surface adhering microbes
15. Remote sharing of data; telepresence
16. Research on rock materials using BH testing procedures
17. Cognitive 'protheses" – nanobots in diagnostics
18. Combined BSL and cleanroom (i.e., PPL) capability
19. Appropriate protective gear for staff working in PPL environment.

APPENDIX A

A DRAFT TEST PROTOCOL FOR DETECTING POSSIBLE BIOHAZARDS IN MARTIAN SAMPLES RETURNED TO EARTH

Introduction to the Draft Protocol

For upcoming Mars sample return missions, NASA is committed to following the recommendations developed by the Space Studies Board (SSB) of the National Research Council (NRC) in its report on sample handling and testing *[SSB 1997].* In particular, the NRC recommended that:

> *a) "samples returned from Mars by spacecraft should be contained and treated as potentially hazardous until proven otherwise," and b) "rigorous physical, chemical, and biological analyses [should] confirm that there is no indication of the presence of any exogenous biological entity."*

To develop the requirements for sample hazard testing and the criteria for subsequent release of sample materials from containment, NASA's Planetary Protection Officer convened the Mars Sample Handling Protocol Workshop Series (MSHP) held over the time-period from March 2000 to June 2001. In addition to U.S. and international participants invited by NASA, significant participation and support by French scientists was provided through arrangement with the Centre National d'Études Spatiales, who participated in all aspects of the Workshops. The stated objective for the Workshop Series was:

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

Therefore the MSHP Workshop Series[33] was designed to devise a protocol that could rigorously analyze returned martian sample material(s) to determine that those material(s) are free from biohazards and/or and extraterrestrial life-forms and are therefore safe to be released from containment in their native state for further scientific research. To accomplish this, participants focused on a variety of questions such as: "What types/categories of tests (e.g., Biohazard (BH), Life Detection (LD)) should be performed upon the samples? What criteria must be satisfied to demonstrate that the samples do not present a biohazard? What constitutes a representative sample to be tested? What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what Physical/Chemical (P/C) analyses are required to complement biochemical or biological screening of sample material? Which analyses must be done within containment and which can be accomplished using sterilized sample material outside of containment? What facility capabilities are required to complete the protocol? What is the minimum amount of time required to complete a hazard-determination protocol? By what process should the protocol be modified to accommodate new technologies that may be introduced in the coming years (i.e., from the time that a sample receiving facility would be operational through the subsequent return of the first martian samples?) The Working Draft Protocol, as a composite, is intended to incorporate the answers developed to those questions.[34]

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33. Appendix F includes citations for all the workshops and reports contributing to this Working Draft Protocol.

34. The reader is referred to the final reports from the prior Workshops in the Series for full documentation of the detailed discussions held by the Sub-groups in those Workshops. As a framework and proof-of-concept, the Working Draft Protocol is a distillation of those discussions and therefore does not include the level of detail brought out in those discussions.

To keep the Workshop Series focused, a set of basic assumptions (see Appendix B, page 133) were given to the participants at each of the Workshops to guide and constrain their deliberations. Subsequent to the failure of the Mars Surveyor 1998 missions, these assumptions were subject to some modification during the re-planning process that NASA and its international partners undertook (note italicized item in assumption #2, Appendix B), however, none of the modifications affected the basic premises under which the Workshop participants undertook their task. These assumptions are consistent with the plans of NASA and its international partners as of the publication of this report.

In addition to the development of this Working Draft Protocol through the NASA-led Workshop Series,[35] in early 1999 the Space Studies Board was asked by NASA to develop its own recommendations for the containment and certification of martian samples – both as input to the NASA Workshop Series, and as recommendations to NASA to be assessed in their own right. Their report *[SSB 2002]* was released in preliminary form in May 2001, just prior to Workshop 4 therefore this Working Draft Protocol also reflects, to a great degree, the findings and recommendations of the Space Studies Board study on this subject.

Why a 'Draft' Protocol?

What is reported here is termed a 'Draft' Protocol because it is and is intended to be just that. While it is a responsibility of NASA's Planetary Protection Officer *[NASA 1999]* to prescribe "standards, procedures, and guidelines applicable to all NASA organizations, programs, and activities" to achieve the policy objectives of NASA's planetary protection program, including ensuring that the Earth is "protected from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from another planet or other extraterrestrial sources," (in this case, Mars), it is neither practical nor useful for this Working Draft Protocol to be developed into a final form at this point in time. On one hand, the final protocol that guides the process of assessing the martian samples should owe much to new knowledge about Mars that will be gained in robotic surface operations on Mars leading up to the sample-return mission, as well as to detailed information available only on the sample-return mission itself. On the other hand, the final protocol should take into account the *specific* nature of the receiving facility that is developed for the initial processing and testing of the returned samples, as well as the requirements and abilities of the *specific* instrumentation and personnel finally selected to undertake the challenging task of testing the samples to protect the Earth from possible hazards, while preserving the scientific value of the sample-return undertaking.

Accordingly, this Working Draft Protocol is intended to provide a proof-of-concept model of the final protocol, demonstrating one approach (and more importantly, a sufficient approach) to testing returned Mars samples for possible biohazards or exogenous biological activity. This Working Draft Protocol has been developed to provide a series of tests that can be applied to martian samples to provide data to address stated criteria for the release of un-sterilized samples from containment – either wholly or partially – while allowing for the earlier release for samples subjected to a decontamination process, to ensure they are safe for analyses outside of containment.

35. This Working Draft Protocol is a compilation of input from all 5 Workshops in the MSHP Series. Prior to Workshop 4, materials developed in all the earlier Workshops in the Series were compiled into a version of the Draft Protocol subsequently designated the "Penultimate Working Draft Protocol." That Penultimate Working Draft Protocol was distributed to the Sub-groups of Workshop 4 and they were asked to refine and finalize it. The Working Draft Protocol is the result of incorporating the comments and changes from the Workshop 4 Sub-groups into the Penultimate Working Draft Protocol (see the Preface for a list of all the versions of the Draft Protocol).

Containment in the Sample Receiving Facility and Elsewhere

The capability is required to handle and process Mars samples to prevent their terrestrial contamination (i.e., clean-room conditions) while maintaining strict biological containment. This requirement is a major challenge in the design of a Sample Receiving Facility (SRF),[36] and to some degree is likely to constrain the working space inside a SRF even more than might normally be experienced in a "typical" Biosafety Level 4 (BSL-4) facility of similar size. An SRF will require an amalgam of technologies currently found in maximum containment microbiological laboratories (e.g., BSL-4, BSL-3) and in clean-rooms used to preserve the pristine nature of rare samples. Such an integrated facility is not currently available anywhere. Some of the design challenges may be alleviated through a design and development process that will include mock-ups of containment/clean-room combinations whose efficacy can be tested thoroughly. Some of the space constraints may be lessened through the use of multiple containment facilities to accomplish different aspects of the final protocol. It is anticipated that samples will be able to be shipped among appropriate containment facilities wherever necessary, and under procedures developed in cooperation with the U.S. Centers for Disease Control and Prevention, the U.S. Department of Transportation, and appropriate international authorities. Nonetheless, it is envisioned that all samples initially returned from Mars will be placed in a single SRF and held there through the preliminary examination phase, and for those subsequent steps compatible with SRF design and capacity.

BSL-4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infection and life-threatening disease. The unknown nature of any possible biohazard in returned martian samples demands, at least initially, the most stringent containment presently afforded to the most hazardous biological entities known on Earth. In the biomedical community, Biohazard testing is a pathway towards gradual "decontainment" of dangerous and exotic bioagents when supported by experimental evidence. Decisions about the appropriate biosafety level for a particular bioagent can be made when sufficient data are obtained to support either the need for continued work at a high level of containment or allowance to conduct work at a lower level.

Generally, lower biosafety levels are assigned to agents with less human virulence. If sufficient data are gathered to rule out concerns about human virulence and infection, a decision could later be made to allow subsequent work at a lower containment level during tests investigating possible environmental effects. A lower level of containment would potentially enhance sample access within the scientific community while still providing adequate biosafety conditions.[37]

In addition to blending biosafety and cleanliness needs, the SRF will need to provide different types of laboratory environments for carrying out the various aspects of protocol testing. During the Workshop Series, a new term 'Planetary Protection Level' (PPL) was developed to be used for the purpose of

36. A variety of names have been used in reference to the place where returned samples will initially be handled and tested (e.g. Sample Receiving Facility (SRF), the Quarantine Facility, the Mars receiving laboratory, primary containment facility, quarantine facility, etc.). A recent NRC report *[SSB 2002]* has used "Quarantine Facility," but it is more useful in this report to use the generic SRF. The actual name and location(s) of the facility or facilities where the protocol will be executed is TBD, though its use beyond the receipt of martian samples may be anticipated.

37. "BSL" levels are a North American convention. European equivalents will be considered and described as necessary in implementation of the final protocol.

categorizing and describing the different combinations of containment and cleanliness conditions required within the SRF for different testing needs. Although details of various PPL designations will require further definition, it is possible to anticipate a number of laboratory conditions that may be required during the protocol testing. The four PPLs are described here and in Table WDP-1.

- PPL-α – for incoming samples and archived samples; maximum biocontainment and cleanliness; maintains samples in an inert gas environment and Mars-like conditions.[38]
- PPL-β – maintains maximum biocontainment and protection for workers and the environment; maximum cleanliness, but allows exposure to ambient terrestrial conditions.
- PPL-γ – maintains maximum biocontainment with moderate cleanliness and ambient terrestrial conditions (i.e., for animal testing scenarios).
- PPL-δ – maintains "BSL-3-Ag" containment conditions with less emphasis on cleanliness and ambient terrestrial conditions.[39]

PPL-type	Biocontainment	Cleanliness[40]	'Ambient' Conditions	Used For:
PPL-α	Maximum (BSL-4)	Maximum	Mars-like (pristine) *Although at 1 ATM w/inert gas environment*	Incoming Container and materials; some preliminary tests; sample bank/storage; some Life Detection.
PPL-β	Maximum (BSL-4)	Maximum	Earth-like	Life Detection; some Physical/Chem; TBD
PPL-γ	Maximum (BSL-4)	Moderate	Earth-like	Some Biohazard, some Physical/Chemical tests, and animal testing
PPL-δ	Strict BSL-3-Ag	Ambient	Earth-like	Some 'post-release' tests TBD

Table WDP-1. Anticipated Laboratory Conditions and PPL Types.

It is important to note that regardless of cleanliness requirements or ambient conditions, all initial testing will be done under maximum biocontainment equivalent to United States BSL-4 *[CDC-NIH, 1993]*. In addition, Biohazard testing will not require the extreme cleanliness levels to be used for

38. It is anticipated that only the primary SRF will be required to have PPL-α conditions. If other facilities beyond the SRF are used as part of the protocol testing, they will be certified for conducting particular tests or studies at the appropriate PPL conditions.

39. PPL-δ provides a level of containment for the samples that allows investigators to work with samples in a laboratory situation that provides protection to personnel through an engineered environment providing HEPA filtered air entering and leaving the area, containment of water and/or waste to the laboratory and protection to personnel through personnel protective equipment consistent with standards U.S. BSL-3 Agriculture and French P4. It was recommended that BSL-3-Ag facilities should be built around large instruments, rather than miniaturizing instruments to fit into a pre-existing lab.

40. Note: Levels of cleanliness associated with each PPL type are TBD and should be defined explicitly and well in advance of sample return.

initial sample processing, or certain P/C or LD tests. The majority of biohazard tests will be done in PPL-γ. If results of initial BH tests and LD tests are all negative, it may be appropriate to conduct some subsequent tests under less strict containment conditions. The first step in downgraded containment for untreated samples has been designated as PPL-δ, which is equivalent to BSL-3-Ag.[41]

"Sterilization" of Martian Samples

Recognizing that a species' adaptation to physiological stress may evolve through natural selection, it is expected that possible extant life on Mars could be able to survive extremely hostile conditions. Surface temperatures at the equator of Mars range from –100°C during the martian winter to 20°C during the martian summer. Mars is extremely dry; the partial vapor pressure of water on the surface is approximately 0.1 Bar. The martian atmosphere is 95% CO_2 and provides no protection against exposure to 200-300 nanometer ultraviolet light, which may generate strong oxidants in the surface material. It is believed that organic compounds on the surface of Mars are subject to oxidation by this UV-induced photochemistry. Since this combination of conditions cannot be found anywhere on Earth, it is unlikely that a single terrestrial species will be found that can serve as a surrogate for a putative martian organism when evaluating methods for sterilizing martian samples. There are terrestrial environments, however, that are sufficiently similar to the martian environment to allow the isolation of species that exhibit extreme resistance to a subset of the conditions (e.g., desiccation radiation, or cold) to be encountered on Mars. As an item for further research, it is anticipated that an effort will be made to identify and characterize terrestrial species from environments as similar as possible to those on Mars, and that these species will be used to validate sterilization processes.

In the context of this Working Draft Protocol and the relevant NRC reports *[SSB 1997; SSB 2002]*, the term "sterilization" may be used to connote the decontamination process that is used to ensure that the samples are safe for analyses outside of containment. It is possible, though very unlikely, that martian organisms are not carbon based, and martian biology could conceivably be based on other elements (e.g., Si, N, P, O, H, S, Al, B). But overall, it should be noted that the chemical elements on Mars and the forces holding molecules together are the same as on Earth. If there were a life-form on Mars based on other than carbon-containing molecules, the energies holding such molecules together would not be much different than those for proteins and polynucleotides. Hence, bond breakage by heat or gamma radiation should be similar for Earth and Mars life-forms, and sterilization conditions for Earth microorganisms should eradicate microorganisms of similar size from Mars. There is no absolutely optimal approach to decontamination under these conditions, but enough is known about the relationship among organism size, repair mechanisms, and survivability, that the maximum survivability of any martian organisms can be estimated with some confidence.

So whether we assume that life on Mars is based on the same building blocks as terrestrial life, or on other covalently bonded complex molecules, only two methods of sterilization are considered viable options at present – dry heat and gamma radiation, either alone or in combination. These methods will penetrate the sample and, therefore, provide the highest level of assurance that putative

41. PPL-δ applies at the point in the protocol where samples do not require atmospheric isolation and may be moved to outside laboratories with suitable facilities for further testing. In general, level 3 biosafety laboratories (BSL-3) abide by different standards within the U.S. and Europe. For clarity, the U.S. standard for BSL-3-Ag will be used.

organisms will be destroyed. It is recognized that the application of heat, and in some cases gamma irradiation, will modify the geological properties of the sample. Within reason, every effort should be made to develop and implement a method of sterilization that protects the scientific integrity of the sample.

Many of the key parameters measured by geochemists are unaffected by sterilizing gamma doses. *[Allen et al., 2000]* Gamma photons from ^{60}Co (1.17 – 1.33 MeV) in doses as high as 30 Mrads do not induce radioactivity in rock and mineral samples. Such doses also produce no measurable changes in isotopic compositions, elemental compositions, or crystallographic structures. The only detectable effects are changes in albedo, color, and thermoluminescence in selected minerals. Isotopic and elemental compositions will not be affected regardless of gamma dose. Sterilization at doses significantly above 30 Mrads may induce changes in crystallographic structure (caveat: research required) and dose-dependent changes in albedo, color and thermoluminescence are expected. On balance, if samples returned from Mars require biological sterilization, exposure to gamma rays or high-energy electrons may provide a feasible option.

For the development of a final protocol for use with martian samples, a program of research should be initiated to determine the effects of varying degrees of treatment by heat and by gamma irradiation on organic compounds in rocky matrices, and also on microscopic morphological evidence of life. This research should be started well in advance of the return of the Mars samples, so that the decontamination process can be designed to allow data obtained from analyses of sterilized samples to be interpreted with minimal ambiguity. Research should also be conducted to determine the efficacy of various supercritical fluids and commonly used organic solvents in killing model microorganisms, allowing the possibility that solvent extracts might be safe to remove from containment without the damage to dissolved biomarker compounds that would be caused by heat or ionizing radiation. Whether or not decontamination is systematically achieved by any supercritical fluids used in making extracts is a matter that must be investigated further, prior to the removal of any such samples from the SRF.

The aim of a sterilizing process is to reduce the risk of significant adverse effects of samples distributed to the scientific community. These levels are defined to be such that the likelihood of exposure to adverse effects for humans, animals, and the environment is less than 10^{-6}. A suggested process for sterilization consists of irradiation with gamma rays at temperatures up to approximately 105°C. This procedure has the advantage of being able to kill all known terrestrial organisms, while doing minimal damage to the non-biologic constituents of the Mars samples.

The survival rate of a large number of terrestrial organisms exposed to ^{60}Co gamma rays has been determined as a function of dosage, dose rate, and temperature. There are no terrestrial organisms known whose probability of survival is $>10^{-6}$ at a dose of 20 Mrads at room temperature. Nonetheless, populations of individual organisms may require higher doses to ensure that the probability of finding any survivor is $<10^{-6}$. The survival rate at a given total dose decreases with increasing temperature during irradiation. For example, the sensitivity of dry T1 bacteriophage to inactivation by X-rays increases, or the D_{37} decreases by ~10-fold between 60 and 105°C *[Pollard 1953]*.

If martian organisms returned to Earth are similar to terrestrial organisms, a dose of 20 Mrads at 105°C should reduce their number to $<10^{-6}$ of their initial number. It is not clear, however, that martian organisms should be similar to terrestrial organisms. It is possible, for instance, that they could be much more resistant to gamma radiation. A good deal is known about the relationship between the size and the biochemistry of terrestrial organisms and their resistance to gamma radiation. It has been shown that smaller organisms tend to survive higher radiation doses, and the strategies used by microorganisms to increase their resistance to radiation are not all understood. It might therefore be a useful exercise to explore hypothetical possibilities for the evolution of martian organisms adapted to the much higher radiation fluxes to which they would be subjected, compared to terrestrial microbes. The radiation dose at various temperatures required to reduce the probability of the survival of a single organism below 10^{-6} per sample could then be estimated and could become the basis of irradiation protocols for the sterilization of returned Mars samples.

Protocol "Sterilization" Conditions – First, we note that a large number of geochemical tests will be carried out in the SRF upon arrival of the samples. These tests will likely include X-ray tomography to determine loci of cracks and other separations where life-forms most likely would be, and Total Organic Carbon (TOC), which permits a limit on the density of carbon-based organisms. Irrespective of the chemical basis of any life-form, a confidence level of sterilization can be provided, with only two assumptions: 1) Any reproducing life-form must be based on macromolecules (i.e., polymers) with interatomic covalent bonds (not crystal lattices), and 2) Since all such bonds have similar strength, destroying these bonds destroys the life-form.

Evidence shows that 55 Mrads radiation will destroy almost all known bacteria, viruses, spores, and prions (e.g., causative agent of Scrapie) by 1 million–fold. Using 100 Mrads would give a 10–fold safety margin. If worst-case estimates are used (10^{6} –10^{12} organisms/gram of martian sample and a tiny target, such as a virus) sterilization would require 400 Mrads. Even this may be satisfactory for most geologic studies (100 Mrads appears to be OK). This amount of radiation could be safely reduced if the irradiation were carried out at elevated temperature (e.g., 105°C), and/or if the TOC is low enough to rule out large numbers of organisms being present in the sample.

Criteria For Release

As part of the charge to the recent NRC study of *The Quarantine and Certification of Martian Samples [SSB 2002]*, the Committee on Planetary and Lunar Exploration (COMPLEX) was asked to study "What are the criteria that must be satisfied before martian samples can be released from the facility?"

The following statements and recommendations were contained in the report of the Committee:

> "COMPLEX considered the possible results of initial searches for evidence of life in the martian samples, especially analyses of the samples for total organic carbon. The Committee's ***Recommendation*** is:
>
> - If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, release of unsterilized aliquots of the samples for study beyond the confines of the Quarantine Facility is justified."

- If the samples contain evidence of life, or if evidence of life is equivocal (*e.g.*, organic matter is present), aliquots that have been sterilized by heat and/or gamma radiation to levels more than adequate to kill any known terrestrial organism (Chapter 5) can be certified for release from the Quarantine Facility."
- If the samples contain evidence of life, or if evidence of life is equivocal, removal of unsterilized aliquots from the Quarantine Facility for transfer to approved containment facilities elsewhere should not be excluded, on the condition that containers and transfer procedures conform to protocols established by a panel of experts (*e.g.,* from the Center for Disease Control) in containment."

and also,

> "The possibility that the martian samples will contain unequivocal evidence of life is very remote, and for this reason COMPLEX's response has been based on the far more likely contingency that evidence of life will be equivocal or absent altogether. Unequivocal evidence of life would dictate a very elaborate plan of handling, curation, and study, which COMPLEX has not attempted to develop."

Recommendation

- "If unmistakable evidence of life is found in the Mars samples, they should be dedicated to biological studies. Studies of the biosignatures in them should be minimal until an optimal study plan has been developed, and an appropriate research facility set up and staffed. In the interim, no aliquots of the samples should be released from the confines of the Quarantine Facility unless warranted by ongoing biological studies, and the samples are sterilized."

as well as,

Recommendation

- "In the likely event that initial examination of the Mars samples can neither prove nor definitively rule out evidence of life in them, plans should be in place to promptly sterilize aliquots of the samples and remove them from the Quarantine Facility for biological and geochemical studies in specialized laboratories elsewhere. This action should not be deferred pending some hypothetical future resolution of the question of whether the samples contain life or artifacts of life."

In addition, a footnote in the report *[SSB 2002]* states that, "The word 'life,' when used in the context of martian life, should always be understood to mean 'Life as we know it,' to allow for the possibility of life-forms distinctly outside our terrestrial experience."

This is an important footnote, because it allows for a possibility that is not, in fact, accounted for by the release criteria that COMPLEX stated in the first recommendation quoted above. It may, in fact, be quite likely that life we may find first on Mars should be "life as we know it," yet there is no assurance of that.

Additionally, COMPLEX's recommendations place a heavy emphasis on "sterilization" of Mars samples as a key to their release prior to "some hypothetical future resolution" of the question of their containing life – yet the report states in a number of places that the effects of sterilizing doses of heat and/or gamma radiation on the geochemical and biological signals the samples may carry are not known. As a result, the release criteria listed in the Working Draft Protocol are slightly more stringent, as well as somewhat more comprehensive, than those recommended by COMPLEX.

Table WDP-2 below gives the basic overview of the questions that need to be answered prior to the release of un-sterilized samples from the SRF. These questions will be asked of a representative sub-sampling of the material returned from Mars.

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

Table WDP-2: Sequence of Questions and Possible Strategies for Decisions about Release of Sample Material from Containment.

In any event, only evidence of real, measurable, biohazards or genuine, active, martian life-forms or their biomaterials should be regarded as relevant criteria for decisions about releasing or not releasing any un-sterilized samples. Depending on results of biohazard and life detection tests, remaining portions of samples will either be released for allocation outright, or sterilized and then released for allocation. Hence, the following criteria are intended to govern the release of samples evaluated using this Working Draft Protocol.

Sample Release Criteria:

- No solid sample shall be released from containment in the Mars receiving laboratory until it or its parent sample undergoes preliminary examination, baseline description, cataloguing, and any necessary repackaging.
  - > Samples to be used for life detection procedures or to be released from containment will be screened for:
    - + Radioactivity;
    - + Potential chemical hazards;
  - > Additionally, samples to be used for biohazard testing will be screened for:
    - + Known toxicity to bacterial and eukaryotic cells;
- Samples containing any genuine active martian form of life, *be it hazardous or not*, will be kept under appropriate level of containment, or be thoroughly sterilized before release.
- Samples providing indications of life-related molecules, including proteins, nucleic acids, or molecular chirality will require more extended testing, including additional Biohazard testing, prior to their release.

- Samples may be released if they are first subjected to a sterilizing process involving heat, radiation, or a combination of these agents to ensure they are safe for analyses outside of containment. A sample that is 'safe' is stipulated to be free of any viable self-replicating entities or entities able to be amplified.
- Samples may be released if Biohazard testing does not yield evidence of live, extraterrestrial, self-replicating entities or entities able to be amplified and capable of propagating on Earth (and possibly interacting with people, animals, plants, or microbes).
  - > Biohazard testing will involve assays for:
    - + Replication in enriched media (liquid/solid);
    - + Effect/growth on various cell cultures;
    - + Effect/growth on whole organisms (i.e., murine/specified rodent; plant); and,
    - + Effect on the ecosystem level.
  - > Basic Biohazard testing will be required even in the absence of evidence of organic carbon in a sample returned from Mars.

Overview of the Draft Protocol

The Mars Sample Handling Protocol has one basic purpose – to ensure that a representative set of sub-samples undergoes sufficient testing to evaluate them against the release criteria. Samples must be characterized, categorized, and analyzed to ensure that they can be sorted according to a procedure providing 'statistical relevance' to any sub-sampling (whether homogenized or pre-sorted for 'biologically interesting features'), and then to test them within a reasonable time using a minimal amount of sample. Early results from Biohazard testing will need to be screened to ensure that potentially chronic effects are not overlooked, and the tests themselves need to be ordered to take into account the relative harm posed by a potential biohazard (e.g., to humans, animals, environments) and to consider a variety of routes of exposure and infection. Samples must be tested for biomolecules (known or suspected), other organic compounds, and for non-carbon evidence of an active metabolism being present. Life Detection and Biohazard testing partially overlap, and both will depend on the processing of the samples and data from the Physical/Chemical processes to evaluate their results and how to seek them.

The Working Draft Protocol has three main segments: Physical/Chemical Characterization, Life Detection, and Biohazard testing. A simplified overview of how the segments are related is given in Figure WDP-1. The overall process of testing can be summarized as the following basic series of steps: first, the sample(s) will be removed from the return container and documented under maximum biocontainment gloveboxes filled with an inert gas atmosphere and housed within a combination clean room/biosafety lab. Following the initial documentation, samples will undergo preliminary characterization, splitting, and detailed examination using a variety of different methodologies. Ultimately, data from Life Detection and Biohazard testing will be used to determine whether or not to release materials from biocontainment. All sample materials not selected for further testing will be archived in sealed containers in an inert atmosphere glovebox within the lab and reserved for future scientific purposes.

The Working Draft Protocol also addresses issues related to facilities, personnel management, monitoring, contingency planning, decision-making, protocol review, implementation, and approval processes.

Physical/Chemical Processing

The overall objective for Physical/Chemical (P/C) processing is to specify information about the samples that will be required to enable effective Life Detection, Biohazard testing, and curation. The focus will be on sample characteristics that could be determinative in understanding the results of both the *in vitro* and *in vivo* testing that may be required, as well as for sample preservation purposes.

P/C processing includes actions affecting the returned samples between the time the SRC arrives in the SRF and the time that sample aliquots are apportioned for Life Detection and Biohazard (LD/BH) tests. Physical and chemical processing should include only those actions required in support of planetary protection and future sample utilization. The details of the proposed P/C processing, which draws heavily from protocols proposed or used by others,[42] is outlined in Figure WDP-2 on page 85.

Principles: The selected steps and investigations in the P/C processing tracks are motivated by the following principles as functions of the SRF: know what the returned samples are, preserve sample integrity, document everything, anticipate that different types of samples (e.g., gases, fines, rocks and cores) require different treatment, recognize that all data obtained in the P/C processing must serve later scientific investigations, use the minimum sample possible, and provide real-time guidance and adjustment to the process. These principles, initially outlined by the report of the Mars Sample Handling and Requirements Panel (MSHARP) *[Carr et al., 1999],* have been endorsed by all the Mars Sample Handling Protocol Workshops *[Race and Rummel, 2000; Race et al., 2001a; Bruch et al., 2001; Race et al., 2001b].*

The first two principles (know the sample; preserve sample integrity) are, to some extent, inconsistent because every characterization method or action on the returned samples will affect them in some way. This inconsistency has been addressed in two ways. First, all initial characterization procedures in P/C processing are nominally non-contact and non-destructive – all the sample mass remains in the same physical and chemical state after each analysis. Second, most of the returned sample is subjected to only minimal investigations, while only a representative portion of the sample is subjected to more specific (and potentially sample-altering) analyses. The P/C processing and screening methods, except for weighing, involve sample interactions with electromagnetic radiation, principally near-visible wavelengths (near ultraviolet, visible, and near infrared). Several methods use X-rays to probe the samples, but it was recognized that X-rays can (at some dosages) affect biological/organic systems.

42. This Working Draft Protocol is based on the framework previously developed by sub-groups at the first Workshop in this Series *[Race and Rummel, 2000, p. 14-19],* and on an earlier report by MSHARP *[Carr et al., 1999],* which are in turn based on the protocols developed at Johnson Space Center for handling and processing of Apollo lunar samples, Antarctic meteorites, and cosmic dust. During various Workshops in this Series, modifications to the draft protocol have been suggested by various sub-groups *[Race et al., 2001a, 2001b],* and several of those have been included here. The present Working Draft Protocol does include several significant differences from the framework developed in the first Workshop in this Series *[Race and Rummel, 2000],* which are duly noted. In general, the Working Draft Protocol is consistent with the requirements and conditions set forth by the Space Studies Board *[SSB 1997],* MSHARP Committee *[Carr et al., 1999],* an earlier workshop on sample quarantine protocols *[DeVincenzi et al., 1999],* and CAPTEM *[Neal, 2000].*

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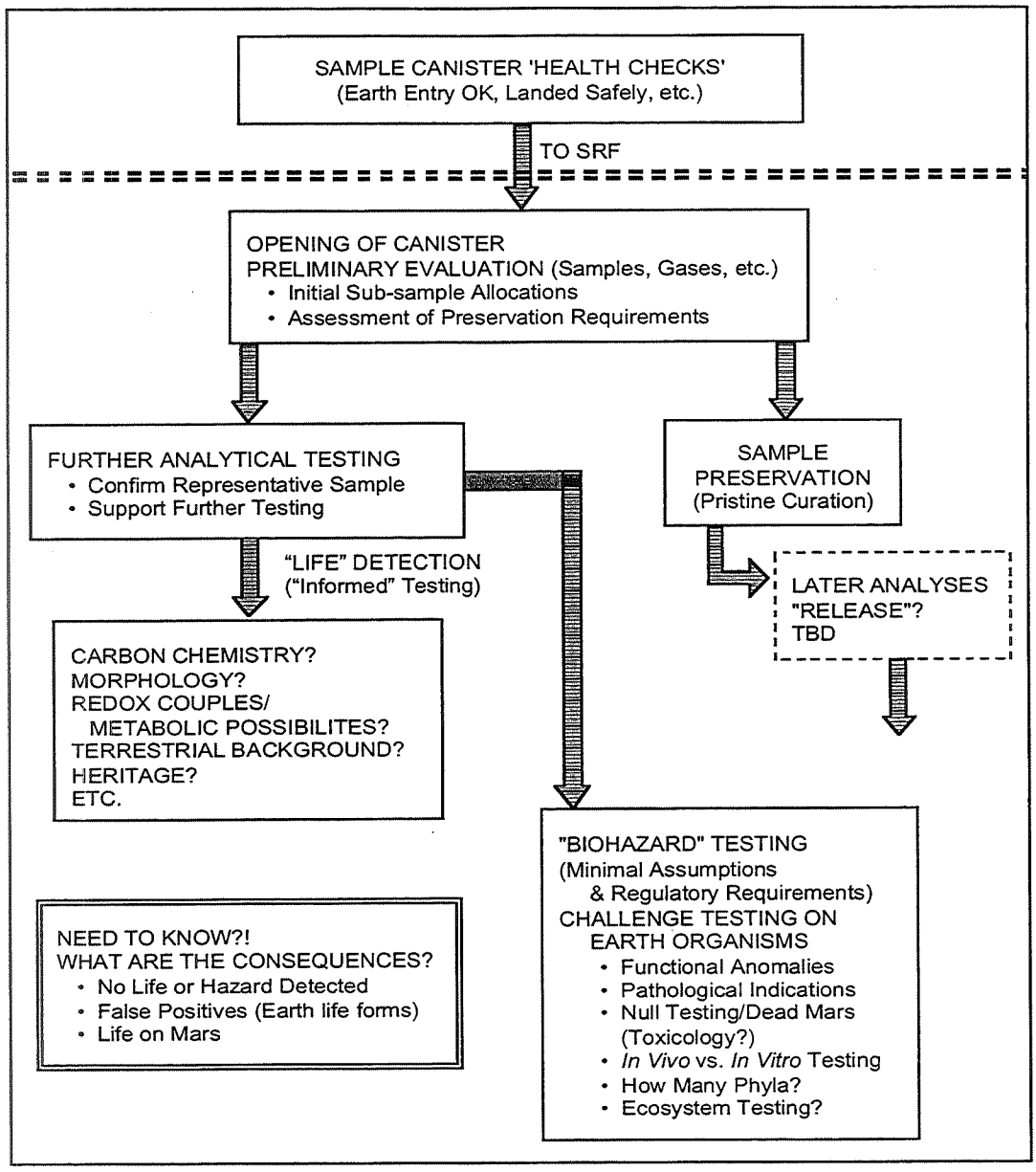

Figure WDP-1: A simplified overview of the Working Draft Protocol showing the 3 main segments: Physical/Chemical, Life Detection, and Biohazard testing.

This Working Draft Protocol attempts a compromise between the desire to affect only a small proportion of the returned sample by planetary protection testing, and the need to assure safety by testing all portions of all samples. A range of strategies have been advocated to deal with the sample testing issue: from "characterize everything with all available non-destructive methods," to "store most of the sample uncharacterized, and do only the minimum with the rest" (see discussions in *Carr et al., 1999, p. 37; Race and Rummel, 2000, p. 18; Race et al., 2001a, p. 35; Race et al., 2001b, p. 34*). Here it is stipulated that it will be essential to examine all the returned material in at least a

minimal fashion, to confirm spacecraft operations in sample transfer from Mars to the Sample Return Canister (SRC), to correlate returned samples with documentation made on Mars, and to provide enough data to make informed choices about samples for LD/BH analyses. Examining all returned materials in at least a minimal fashion will help avoid a worst case scenario where an obviously biogenic sample could be stored unexamined and only discovered after nominal LD/BH tests were completed.

Documentation: All treatment and actions with the returned samples need to be documented fully. Without a high level of documentation, it would be impossible to establish which samples are representative or particularly interesting, and to indicate what had been done to which sample during processing.

Different Samples: It was clear that the different types of samples will require different processing techniques. Gases and bulk fines samples are expected to be inherently homogeneous to some level, and will require only minimal processing to derive characteristic and representative samples. However, solid materials are anticipated to be potentially heterogeneous and will require more extensive study and real-time decisions about processing.

Minimum Sample Mass: The amount and size of returned Mars samples will be small, and it will be desirable to subject sample materials to a great range of biological, physical, and chemical tests. Thus, by necessity, each test on a returned sample must use the minimum mass consistent with achieving the scientific goal of the test.

Real-Time Adjustments – Oversight Committee: Provisions must be made to adjust the P/C processes in response to changing technology and mission specifics, to monitor the processes in progress, and to adjust them in real time to fit the actual returned samples *[Carr et al., 1999, pp. 7, 9]*. This current Working Draft Protocol is being written thirteen years before nominal return of Mars samples to Earth. We do not know the spacecraft configuration, the types of martian samples that will be collected, their return configuration, and the exact nature of planetary protection measures. Similarly, we cannot anticipate the advances in instrumentation and analytical methods that are likely between now and the time of sample return.

It is likely that the returned samples will not be exactly as we imagine them now, and may include materials that are complex (e.g., breccias) or unusual (e.g., a possible stromatolite fossil). Treatment of these types of samples would be sample-specific, and cannot be defined in advance. Thus, there must be a mechanism like a Scientific Oversight Committee to adjust the final protocol to fit the samples.

Assumptions: In preparing the P/C portion of the Working Draft Protocol, we have assumed the mission profile and constraints outlined in the initial Workshop of this Series *[Race and Rummel, 2000]*. It is worth reiterating a few of the key assumptions with particular relevance to physical chemical processing: the SRCs will be received at the SRF intact and with no breaches of containment; the returned samples will include gas, fines material (bulk regolith), and solids; and total sample mass is expected to be approximately 500 to 1000 grams.

Overview of Physical/Chemical Processing: Physical and chemical processing are the priority actions taken with the returned Mars samples between arrival of the SRC at the SRF and initial examination for biohazard and LD/BH of fines and solids. These anticipated steps in P/C processing are shown schematically in Figure WDP-2 which is based on portions of Figures 6-2 and 6-3 of *Carr et al., (1999),* Figure 1 on page 18 of *Race and Rummel (2000),* and the narrative of *Race et al., (2001a).* The numeric annotations in Figure WDP-2 refer to similarly numbered sections of text below, which elaborates on the proposed P/C processing in narrative form.

P/C processing can be divided into three phases in roughly sequential order:

- Initial pre-processing, before preliminary examination of the samples;
- Preliminary examination and screening of gas, fines, and solids, to permit informed choices about samples for later detailed testing, banking or curation; and
- Sub-division of those samples selected for Biohazard and Life Detection tests.

Following P/C processing, Life Detection and Biohazard testing will begin. Those processes may require information developed during preliminary examination and screening, and may also require subsequent more detailed information of a physical or chemical nature. Analyses to obtain these latter data are supplemental to the P/C processing and are not included here.

The steps of preliminary examination and screening were judged different for three types of samples: gases; homogeneous particulate samples; and inherently inhomogeneous samples like rocks, rock cores, and regolith cores. Each of these sample types will follow a different track through preliminary examination and screening as described in the text below and shown on Figure WDP-2 as the *'Gases Track,' 'Solids Track,' and 'Fines Track.'*

Pre-processing Samples

- *1.0 Pre-Processing Steps:* Pre-processing steps outlined here are those between arrival of the SRC at the SRF, and initial examination of gas, fines and solids. Preprocessing steps refer to cleaning and decontaminating the exterior of any containers holding samples, as well as the initial steps in each of the gases-, fines-, and solids-tracks involving opening of containers and removal of samples.

- *1.1 Clean and Decontaminate Exterior of SRC:* It is imperative that the exterior of the any sample return containers or vessel(s) carry no terrestrial microorganisms and are organically clean. (It is assumed that the exterior of the SRC is not contaminated with martian materials.) If these states are not achieved, all subsequent analyses for life or biohazard are severely compromised. The actual methods of cleaning and decontamination are to be determined. An interesting new method is laser ablation of the SRC exterior.

  Procedures for opening sample containers are mission specific, as to number, types, and contents of containers. At a minimum, we assume that some solid materials with surrounding gas will be in the container(s). It is recommended that the gas be extracted for separate treatment, and that the solid samples be contained thereafter in an inert gas like dry nitrogen.

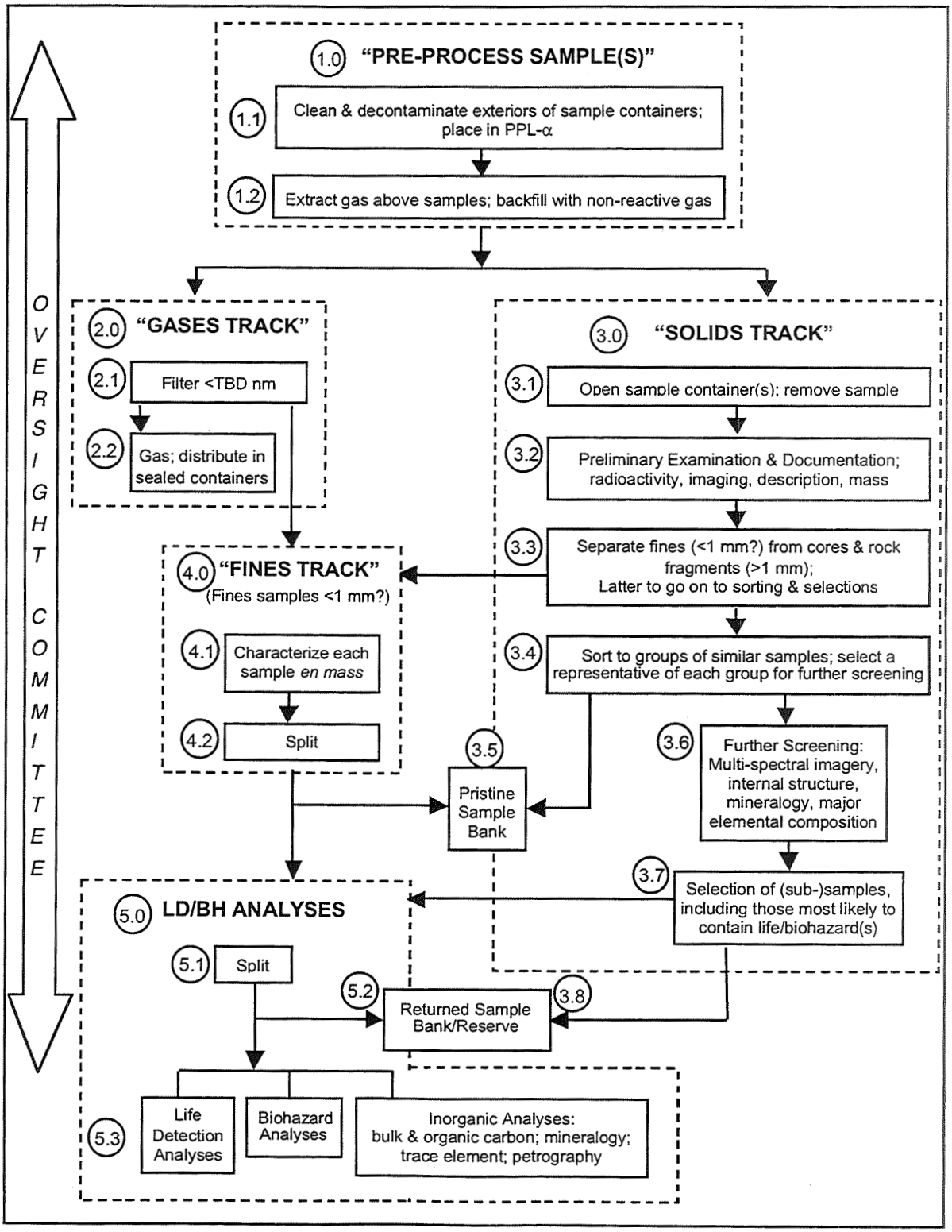

Figure WDP-2. The Physical/Chemical Analyses will occur in four sequential stages leading into the Life Detection and Biohazard testing.
(The numbers in circles correspond to numbered paragraphs in the text).

- *1.2 Extract Head Gas and Back-fill:* The returned solid samples will arrive on Earth with some gas surrounding them. Presumably, this "head gas" would consist originally of martian atmosphere. By the time of arrival on Earth, the gas might have been affected by chemical and physical reactions with the solids (rock and soil), by out-gassing from the solids (especially if the temperature rises above 25°C during return), and possibly by biological activity in the sample. Thus, this gas may contain information important to understanding the thermal, chemical, and biological histories of the solid returned samples. Therefore, extraction and analysis of the head gas is a high priority.

  In this step of pre-processing, the head gas would be extracted from the SRC, and the SRC back-filled with a chemically un-reactive gas to ambient "room" pressure. Exact procedures for extraction and back-filling will depend on the SRC design and construction, but might (for instance) include puncturing the SRC at an intentional thin point, extracting the head gas to a pre-determined vacuum pressure, and refilling the SRC with dry clean N_2 gas. The extracted head gas would be processed as below (see 2.0 – 2.2).

  Three issues related to gases were identified for further consideration and possible research:

  - the effects of vacuum and non-martian gas on the chemical properties of the sample;
  - the effects of vacuum and non-martian gas on any live martian biota; and
  - the effects of extraction on gas isotope ratios.

  For the first issue, experience with curation of the Apollo lunar samples has shown that few geochemical and other inorganic investigations are materially affected holding and processing the samples in dry N_2 gas at 1 bar. Of course, the lunar samples originated at hard vacuum on the Moon. It is not clear, however, what changes might be wrought on returned Mars samples (possibly containing clays or other hydrous materials) by vacuum pumping and then by immersion in dry N_2 gas. This is an area for research.

  For the second issue, there is reason to want the returned solid samples to be treated under atmosphere as near to martian as possible – both to preserve key geochemical signatures *[Neal, 2000, p. 22,492ff]* and to maintain potential micro-organisms in their native environment. No one knows whether live martian organisms could be killed by removal of 0.006 bars of CO_2 and then immersion in one bar of N_2 and there may not be comparable terrestrial biota to test. The samples will eventually be subjected to higher pressures, merely because the biota of BH tests would not survive in martian atmosphere. On the other hand, there are serious problems in sample handling and geochemistry that would be caused by immersing the samples in a model martian atmosphere. Sample handling and LD/BH testing at reduced pressure (the near vacuum of 0.006 bars CO_2) present severe problems. Sample handling under vacuum was attempted during the Apollo program with lunar samples, and was found to be extremely difficult, expensive and contaminating (e.g., mercury or oil from vacuum pumps). Similarly, backfill with a relatively reactive gas like CO_2 will change the isotopic nature of the sample. Terrestrial carbon and oxygen will exchange with the sample and compromise biological and geochemical inferences from of these two stable isotope systems.

  This is obviously an area of future research. One possible approach would be to backfill the SRC and do sample handling and examination (where possible) under 1 bar of dry N_2 gas with 0.006

bars of CO_2 added. This might satisfy the constraints of easy sample handling and the hope of not killing live martian organisms.

For the third issue, it is known that the elemental and isotopic ratios of a gas sample can be fractionated during transfer from one reservoir to another. With the head gas in contact with the abundant surface area of the returned samples, fractionation could become a serious potential problem.

Gases Track

- *2.0 Gases Track:* Gas withdrawn from the SRC, the "head gas," will be processed by filtering. Subsequently, any fines split off for Life Detection and Biohazard testing, and the filtered gas, would be available relatively rapidly for other investigations *[Race and Rummel, 2000, p. 17].*

- *2.1 Filter to <TBD Nanometer:* After or during removal of the head gas from the SRC, the gas should be filtered to remove particles *[Race and Rummel, 2000, p. 17].* The purpose of filtering the head gas is to remove objects that could reasonably constitute viable organisms or that might present biohazards. The size of objects passing the filter is to be determined (TBD). Sizes suggested by previous sub-groups in this Workshop Series have ranged from <0.5 µm *[Race et al., 2001a, p. 34]* to <0.02 µm *[Race et al., 2001b, p. 27],* both of which are realizable with current technology (currently, some methods are rated to remove particles larger than 0.003µm). It is not clear if filtering could change the chemical or molecular composition of the head gas, for instance by preferential adsorption of heavy noble gases or by catalysis of reactions. This is an area for additional research.

- *2.2 Distribute in Sealed Containers:* The Sub-group recommended that filtered head gas could be released from the SRF and distributed in sealed containers. Unlike the returned solid samples (rock, regolith, etc.), a returned gas sample is only useful for investigation if it is contained. Typically, a gas sample like this would be placed in a glass bulb, which would then be sealed by melting the stem of the bulb. Containment at PPL-α or PPL-β levels seems inherent in this procedure,[43] and it is recommended that the filtered gas be available for immediate allocation from the SRF without further processing or sterilization.[44]

Solids Track

- *3.0 Solids Track:* After removal and filtering of head gas from the SRC, the remaining returned samples would be solids of various types: regolith samples, rocks, rock cores, soil cores, and fines. The specifics of this solid sample set are to be determined during mission design. These solid samples will be processed through two separate tracks, Solids Track (3.0) and Fines Track (4.0), for basic documentation, further preliminary testing, and selection for subsequent Life Detection and Biohazard tests.

43. It is assumed that the operation of sealing the gases into the bulbs will be done under appropriate PPL conditions.

44. To date, no decisions have been made about when and under what conditions sample materials will be eligible for release from containment at the SRF. Ultimately, it is likely that decisions about what is done with sample materials will be made after review by an appropriate international scientific oversight committee at the SRF in consultation with NASA's Planetary Protection Officer and other responsible officials.

Some principles of this P/C process are worth restating here. The P/C process is a method to obtain the minimum data needed to adequately characterize the samples and to permit selection of suitable samples for LD/BH tests. The remaining samples would be preserved and made available for subsequent investigations and analyses. The samples will be changed from their original state as little as possible.

The martian samples will be touched or come in contact with only a limited set of materials under controlled temperature and atmosphere. Pristine lunar samples are touched only by stainless steel, aluminum, and Teflon™; these might also be suitable for returned Mars samples. Neal cites the considerations *[Neal, 2000],* from a geochemical perspective, for choices of materials for sample handling and suggests several types. Whether these materials are appropriate for returned martian samples should be determined through additional research with Mars simulants prior to sample return.

The temperature of processing is to be determined, and will depend in great part on technical mission constraints. The implicit assumption here has been that temperature of processing will be between 0°C (273K) and ambient (~298K), for which the protocols and experience with the Apollo samples are relevant. On the other hand, it would be important from geochemical and biological perspectives to maintain the returned sample at its ambient martian temperature, ~240K *[Carr et al., 1999; Neal, 2000].* This temperature may not be possible within mission constraints, and there appears to be no compelling reason to process at temperatures significantly below those experienced by the samples during their transit to Earth. It is not clear, at this point, what problems and attendant costs would be associated with sample curation and processing at sub-freezing temperatures.

It is suggested that processing, curation, and back-filling of the SRC be performed at an atmosphere of 1 bar of un-reactive gas; the composition and pressure of the atmosphere has implications for biological and geochemical testing, and is an area of concern (see pages 33). The following steps implicitly assume that processing and curation will take place under a pure un-reactive gas (such as N_2) at 1 atmosphere of pressure. It is not known whether this gas would present problems to Life Detection and Biohazard testing procedures. It must be recognized that a requirement for processing at low pressure, like the 0.006 atmospheres of the martian surface, would have significant implications for the design and cost of a SRF.

- *3.1 Open SRC and Remove Samples:* The SRC must be opened for retrieval and removal of solid samples from it. The procedure for opening the SRC and removing the samples are to be determined and will depend entirely on the design of the SRC.

- *3.2 Preliminary Examination and Documentation:* As part of the P/C processing, Preliminary Examination and Documentation includes the minimal investigations deemed absolutely critical for understanding the nature of the returned sample, and initial hazard investigations *[Race and Rummel, 2000, pp. 14, 17; Race et al., 2001a, p. 37].*

The sole hazard investigation at this time is measurement of sample radioactivity, because some forms of ionizing radiation can penetrate the curation barriers between the returned sample and human processors. The purpose is not to measure abundances of indigenous radioisotopes

(e.g., ^{238}U) nor cosmogenic radioactivities (e.g., ^{26}Al), but rather to determine whether radiation levels associated with the samples could pose a threat to workers at the SRF. Hazardous radioactivity can be measured on the bulk returned sample, and need not be measured on individual samples unless the bulk presents a radiation hazard. Only gamma radiation need be detected, as beta and alpha radiation will not penetrate the barriers between the returned samples and human processors. In the opinion of the Workshop attendees, it was extremely unlikely that returned martian samples will present a radiation biohazard.

Imaging provides the first and critical documentation of the returned sample *[Race and Rummel, 2000, p. 17].* Imaging at this stage would serve multiple objectives: verification of mission success; correlation of specific samples with images of them taken on Mars and their sources; documentation of physical effects of transport to Earth (e.g., fracturing, disaggregation), preliminary identification of rock types, and measurement of sample volumes. It is anticipated that the returned samples would be imaged at a high spatial resolution (TBD; perhaps ~0.1 millimeter per pixel), in wavelengths to TBD (perhaps approximately seven-to-nine wavelengths, with at least three or four in the visible). These data will be critical to understanding the nature of the returned sample and in processing and selection of samples for Life Detection and Biohazard tests.

Masses of samples should be measured first at this stage, and subsequently whenever a sample is cleaned, split or allocated. Measurement of mass is important as a mission design requirement, for the sample tracking and curation, and for helping allocate suitable samples for LD/BH testing. For instance, it is likely that a mission requirement would be return to Earth of a given mass of martian material, and weighing here will determine if that mission requirement has been fulfilled.

- *3.3 Separate rock fragments and cores from fines:* At this stage of processing, the solid returned sample would be separated into larger and smaller fragments. The former would include drill cores, whole rocks, and rock fragments or rocklets[45] (equivalent to the Apollo "coarse-fines"). The latter would include unconsolidated regolith, atmospheric dust, and dust generated by coring operations. This separation is necessary because the larger fragments cannot be treated as homogeneous powders, and must be examined individually for Life Detection and Biohazard analyses. It is possible that the regolith samples will include small rocks and rocklets, comparable to the case with the lunar regolith samples returned by the Apollo missions. As with Apollo, the small rocks and rocklets would be separated from the finer material, cataloged, and curated individually throughout subsequent processing and analyses. The cut-off size for rock fragments or rocklets remains to be determined. The standard cut-off size in the soil science community is greater than 2 millimeters. Previous sub-groups in the Workshop Series have suggested sizes ranging from greater than 1 millimeter to greater than 2 millimeters, and even "... greater than several millimeters ..." *[Race et al., 2001a, p. 34; Race and Rummel, 2000, p. 17].* It seems reasonable that decisions about cut-off sizes for different classes of solid materials will be made when the sample is returned and first examined, based on a recommendation of a science oversight committee.

45. The terminology used to refer to small rocky materials has varied from workshop to workshop in this Series. The terms rock fragments, rocklets, and pebbles have been used to identify a general class of solid material that is distinct from fines, larger rocks or rock cores. In addition to determining cut-off sizes at some later date, it will be advisable to use consistent terminology in all parts of the protocol.

Given the dusty nature of the martian surface, and the likelihood of dust generated during coring, it is anticipated that the surfaces of cores and rock samples will be coated with fine-grained materials. After separation, preliminary examination, and documentation of the returned solid materials, it will be necessary to remove dust from surfaces of the cores, rocks, and rocklets *[Race et al., 2001b, p. 22].* These fine materials constitute distinct samples of martian material, and will require different processing and curation than the solids (i.e., they will be treated as in the fines track). In addition, the fine materials on solids will likely hinder identification and processing of the latter by obscuring their surfaces. Selection of samples for Life Detection and Biohazard assays will require knowledge of the mineralogy, structure, and textures of the samples. The analytical probes available (primarily visual and near-infrared optics) will be unable to operate effectively on dust-covered samples.

The exact method of fines removal is to be determined. Suggested methods have included vacuuming the samples, blowing the dust off, a combination of vacuuming and blowing, and laser desorption. In all these cases, thought needs to be given to how the fines are to be collected after removal. The fines collected from each solid sample would be identified individually, and treated as a separate fines sample within the "fines track," as described in section 4.0 below.

- *3.4 Sort to Groups:* After removal of adhering fines, the solid samples should be sorted into groups of similar materials using visual clues and information from Preliminary Examination data *[Race and Rummel, 2000, p. 17; Race et al., 2001a].* This step assumes that the returned sample will contain several cores and/or multiple millimeter-sized rock fragments ("rocklets"). Criteria for sorting would include size, rock type (including color), grain size, texture, and other readily observable properties. This sorting is an important first step towards selecting representative samples for Life Detection and Biohazard tests *[Race et al., 2001a, p. 26].*

- *3.5 Pristine Bank:* Samples and sub-samples that are not chosen at this point for *Further Screening* and/or for Life Detection and Biohazard tests will be stored in a *Pristine Sample Bank [Race and Rummel, 2000, p. 17].* This "bank" will serve as a containment system designed to maintain the physical, chemical, and biological integrity of samples while they await allocation for other analyses at a later date. According to recommendations by CAPTEM, the "bank" should hold the samples under an inert atmosphere at temperatures below 240K *[Neal, 2000].* The pristine solid samples are those that have been affected by no procedures beyond those of preliminary examination, dust removal, and sorting. The pristine bank will serve the critical purpose of preserving a portion of the returned sample for analyses beyond and after the Life Detection and Biohazard assays associated with planetary protection. The pristine bank samples will become the principal resource for all subsequent chemical, geological, physical, and biological analyses on the returned samples.

- *3.6 Further Screening:* At this point, sub-samples of each rock type group sorted previously (see section 3.4 above) would be subjected to additional analyses in support of (and preliminary to) Life Detection and Biohazard tests *[Race and Rummel, 2000, p. 14; Race et al., 2001a, p. 37].* The exact analyses needed are to be determined in conjunction with the detailed LD/BH tests which are also TBD (see *Future Research* page 32). Selected analyses should emphasize non-destructive methods that are not likely to modify or destroy biological molecules and biohazards, and would not be anticipated to kill or weaken live martian organisms. Once they are defined, it will

be possible to learn what characteristics of the returned samples would affect or interfere with the tests, and what data are essential prior to the tests. With these data in hand, the *Further Screening* analyses can be tailored to meet the requirements of life and biohazard detection. Given these restrictions and uncertainties, the following screening methods have been suggested.

Multi-spectral imagery of the samples in visible, near-infrared, and/or thermal infrared light will provide identification of the minerals (inorganic chemical compounds) and presence and distributions of organic matter and water (molecular and bound) in the sample. Raman spectroscopy should be considered here also, with the caveat that samples can experience significant heating during Raman analysis. (For instance, 514.5 nanometer green light from an argon laser is absorbed significantly more than 1064 nanometer infrared light from a Nd:YAG laser. Heating can also be mitigated by distribution of laser power in space and time over the sample). The distributions of minerals on the samples' surfaces will be crucial clues to understanding their internal structures. X-ray diffraction analysis would also be valuable in defining the minerals in the samples (see *Race et al., 2001a,* p. 35ff, for more detail on these methods.)

It is important to know the internal structures of the samples (especially the larger ones), because biogenic material could reasonably be concentrated in cracks and open spaces (analogous to terrestrial endolithic organisms). Building on the imagery above, tomographic analyses could provide three-dimensional visualizations of the internal structures of the samples. Among tomographic methods, the most developed at present is X-ray tomography. To provide X-ray tomographic maps of density (i.e., continuum absorption of X-rays) now requires only a bench-top instrument. X-ray tomographic maps for individual elements (like carbon) require at present the X-ray intensity of a synchrotron light source, and is likely impractical in this *Further Screening* step.

Abundances and distributions of major elements and several minor elements will likely be important for sample selection in life-detection and biohazard analyses. It is also possible that abundances of certain elements could produce false positives or negatives on Life Detection and Biohazard tests. A likely method for elemental analysis is X-ray fluorescence, a mature technique used routinely in inorganic geochemistry.

It would be very important at this stage to have bulk analyses for carbon as a guide to sample selection. However, none of the sub-groups in the MSHP Workshop Series suggested a non-destructive test for bulk carbon that was sufficiently precise and had low enough detection limits to be useful here. This is an area for future research.

- *3.7 Selection of Sub-samples:* Based on data from the *Further Screening* tests (section 3.6), representative sub-samples will be selected for Life Detection and Biohazard tests. The remaining unselected samples will be stored in the Returned Sample Bank (section 3.8) for future research access. Selected samples will carry forward to the actual Life Detection and Biohazard investigations (section 5.0).

- *3.8 Returned Sample Bank:* The *Returned Sample Bank,* distinct from the *Pristine Sample Bank* (see section 3.5 above), is for storage of samples that have experienced the analysis of *Further Screening,* but have not yet been allocated for Life Detection and Biohazard tests. These returned

samples should be labeled and kept distinct from the pristine samples, as the former have had more chance for contamination than the latter.

Fines Track

- *4.0 Fines Track:* Fines samples are those with particle sizes smaller than some to-be-determined limit; the size limit suggested by earlier sub-groups in this Workshop Series was 1 or 2 millimeters *[Race and Rummel, 2000; Race et al., 2001a, 2001b].* In either case, it is anticipated that fines samples will contain so many grains, mixed homogeneously, that it will be readily possible to take representative splits for Life Detection and Biohazard tests. Fines samples may include materials from a variety of sources: material collected as such, like dust from a wind-deposited dune; regolith that has had coarser material removed (see section 3.3 above); dust filtered out of the SRC head gas (see section 2.1 above); or particulates removed from surfaces of rocks or cores (see section 3.3 above).

- *4.1 Characterization:* Characterization of fines samples would be limited to imagery of each bulk fines sample (possibly including multi-spectral imagery) and weighing of each bulk sample *[Race et al., 2001a, p. 35].* There is no need to image or otherwise characterize each individual particle within a bulk fines sample. Only these minimal analyses are needed to document each fine sample at this stage in order to select samples or representative sub-samples for biohazard and Life Detection assays. Sub-groups at the first Workshop in this Series suggested that each fines sample be subdivided into fragments larger and smaller than 1 millimeter *[Race and Rummel, 2000],* but this suggestion was not pursued by any later Sub-group. It may be an area of needed research.

- *4.2 Split for LD/BH Tests and Banking:* At this point in P/C processing, fines samples would be selected for Life Detection and Biohazard tests, and split into representative aliquots. Some aliquots would be carried forward to Life Detection and Biohazard tests (see section 5.3 below), and some would be reserved in the *'Pristine Sample Bank'* (see section 3.5 above).

  The methods of splitting the fines samples are to be determined. Methods used in typical terrestrial applications (e.g., riffle splitter or coning-and-quartering[46]), may not be appropriate or practical here *[Race et al., 2001a, p. 14].* First, these methods involve considerable contact between the sample and tools and surfaces, and may be deemed too contaminating. Second, both methods have the potential for considerable loss of sample through embedding in metal surfaces or electrostatic adhesion to metal and plastic surfaces. The electrostatic adhesion problem will be exacerbated in the dry atmosphere of the PPL-α spaces, as has been found with curation of lunar samples. In fact, neither method is now used for splitting lunar fines samples. This is clearly an area for research.

  In this Working Draft Protocol, it is assumed that a sub-sample of fines is representative, based on confirmation of an adequate splitting method. However, previous sub-groups *[Race et al., 2001,*

46. A riffle splitter is a mechanical separation device that is able to split an unconsolidated soil sample into two equal parts that have the same grain size distribution (and presumably composition as the parent sample). Coning-and-quartering is another commonly used separation method (as described in *Maxwell 1968*).

p. 14] suggested that each fines sample be split into multiple sub-samples and each analyzed for bulk composition and mineralogy (as under *Further Screening*, see section 3.6) to determine whether splits are homogeneous. Further consideration of this issue is needed.

Life Detection and Biohazard Analyses

- *5.0 Samples for Life Detection, Biohazard Analyses:* At this point, samples have been selected for Life Detection and Biohazard tests as well as other P/C analyses

- *5.1 Split into Representative Sub-samples for LD/BH:* The samples selected for Life Detection and Biohazard tests will be split into representative sub-samples at this point. This splitting is necessary to ensure that analyses are performed on similar materials, and so that the results of one test may be reasonably correlated with the results of another. Splits chosen for immediate analysis will proceed to various LD/BH analyses (see section 5.3 below). Some splits will be held in reserve as part of the return sample bank as described in section 5.2. below.

- *5.2 Reserve:* Some splits from section 5.1 will be held in reserve for Life Detection and Biohazard tests, in anticipation of future needs. Should a test fail or require repetition, this reserve material would be available. These reserve splits could reasonably be kept in the *'Return Sample Bank,'* but labeled accordingly.

- *5.3 Parallelism of Tasks:* It is beyond the scope of the P/C procedure to describe the actual operation of Life Detection and Biohazard analyses and supporting inorganic analyses. However, they are included on Figure WDP-1 for completeness. It is anticipated that these three types of tests would be run in parallel, with the results of each influencing the interpretation and course of the other tests *[Carr et al., 1999, p. 9]*.

Future Research: In the discussions about physical and chemical processing of the returned martian samples, several areas were identified where data were not available or could readily be obtained without additional research. Each research suggestion discussed below is keyed to the particular narrative text section above where it is called out:

- What analyses and data do the Life Detection and Biohazard analyses require from the physical and chemical processing? (sections 3.2, 3.6, and 4.1). These requirements were not available, so the P/C process here reflects informed judgment (mostly from geochemists and geologists) about which analyses would be most useful in LD/BH studies. In particular, it would be very important to know what information about sample characteristics or the particular P/C processing would be important to know for LD/BH purposes (for example, as possible causes of false positives or negatives); to document abundances of specific elements of interest (e.g., arsenic) or minerals (e.g., saponite clay); or to characterize surface reactivity and constituents (e.g., super-oxidants), etc.).
- In implementing the final protocol there should be close collaboration between biohazard, toxicology, and pathology disciplines with those conducting testing in chemistry, biochemistry, geochemistry, physics, and geophysics to coordinate a truly integrated testing outcome, pursuant to augmenting what physical sciences data should be ruled in or ruled out in ultimate interpretations of sub-sample biohazard and/or toxicity.
- Trial testing initiatives should be developed before the final protocol is fully implemented in a sample return mission. These trials should be refinements that take into account the

prospective chemical and physical properties of martian soil and rock(s) (and/or use martian surrogates where applicable), as well as evaluate biohazard containment facility needs.

- Is there added value in separating each fines sample into grain size separates *[Race and Rummel, 2000, p. 17]*? What additional contamination might be introduced by this procedure? (section 4.2)
- How can one remove terrestrial contaminants (including organics) from the exterior of the SRC before it enters PPL-α space? Laser ablation surfacing was suggested and should be studied. (section 1.1)
- How can one effectively remove dust and other fines from the surfaces of rocks and rock cores? (section 3.3) Three suggestions were vacuuming, blowing with compressed gas, and laser desorption.
- What effects do X-rays have on biological structures and molecules? Several analytical methods involve interaction of X-rays with the samples (e.g., XRD, XRF, XR tomography), and the Sub-group 1 did not know whether these X-ray doses would affect LD/BH analyses. (section 3.6)
- How can one analyze a bulk sample for trace or ultra-trace quantities of carbon, non-destructively and without anticipated deleterious effects on biological molecules or viable organisms? (section 3.6)
- Is the chemical composition of the head gas affected by filtration to remove small particles? (section 2.1)
- How can one produce representative splits of martian dust and fines materials without unacceptable contamination or loss of sample? (section 4.2)
- How can one confirm that splits of dust or fines material are representative before Biohazard and Life Detection analyses, or is such confirmation necessary? (section 4.2)
- What chemical and physical effects would removal of head gas and replacement with dry nitrogen have on the returned martian samples? (section 1.2)
- What chemical effects would removal of head gas from the returned sample canister have on the gas itself? (section 1.2)
- What effects would removal of head gas and replacement with dry nitrogen have on live martian and terrestrial organisms in the returned martian samples? Would these effects be mitigated if samples were curated under dry nitrogen with 0.006 bars of CO_2 gas? (section 1.2)
- What effects would gas with terrestrial carbon and oxygen isotope ratios have on live martian and returned terrestrial organisms in the returned martian sample? Perhaps, would live martian organism ingest the terrestrial carbon and oxygen, and become isotopically indistinguishable from terrestrial organisms? (section 1.2)
- Using Mars simulants, determine whether materials and conditions recommended by CAPTEM *[Neal, 2000]* are appropriate for handling martian samples. (sections 3.0 and 4.0)
- Petrographic thin sections are enormously valuable in characterizing the minerals, structures, textures and history of a rock. Can petrographic thin sections be produced in a manner consistent with the principles of minimal sample use and minimal contamination of the section material and the remaining sample? (section 5.3)

Areas of Concern: Several areas of serious or general concern have been raised during discussions of physical and chemical processing; these issues are significant enough to affect mission design, SRC design, and SRF design:

- The validity and significance of biosafety and Life Detection procedures in the SRF is strongly dependent on sample collection procedures on Mars, and thus on spacecraft and mission

design. How can the biohazard and Life Detection teams have adequate influence on the designs of sample return spacecraft and sample collection procedures?

- What if the return sample container is breached or its seal is compromised? What contingency plans are possible to achieve PPL-α containment and biosafety? (see Appendix B, *Assumptions*, page 133).
- Is measurement of sample mass important as a preliminary characterization step? Should it be deferred until the "Further Screening" step? (sections 3.2 and 3.6).
- How is the head gas to be removed from the SRC without contamination? (section 1.2) Is backfill with non-reactive gas justifiable in terms of possible effects on martian biology? Would it be adequate to backfill with 6 millibar of terrestrial CO_2 and the remainder a non-reactive gas? (section 1.2)
- What should be done if a unique critical sample is smaller than the nominal requirements for LD/BH analyses? (section 3.4 ff)
- What should be done if the requirements for LD/BH testing evolve to consume an inordinate quantity of returned sample, to preclude other biological, organic, and inorganic tests that further NASA's other goals? (section 5.0)
- Although not directly relevant here, concern was expressed that sterilization measures might have significant adverse effects on biochemical analyses outside of PPL containment *[Race and Rummel, 2000]*.

Life Detection

Introduction: The proposed Life Detection (LD) analyses will use a broad definition and set of criteria for life (and an approach for detecting life) that are not limited by the specific features of life as we know it on Earth. The approach should begin with, and rely on 'signatures' of various types that encompass all known terrestrial life, and that might encompass non-terrestrial life. These signatures should be based on macromolecular structures, structural and biosynthetic chemistry, isotopic patterns and geochemical features that help define the underlying principles of life (see *Biosignatures* below). We will take advantage of our knowledge regarding the structural and metabolic intricacies of earthly life, but will not be constrained by these terrestrial examples. In particular, the recent recognition of our inability to cultivate nearly all terrestrial microbial life emphasizes the importance of relying on methods beyond *in vitro* cultivation for detecting extraterrestrial life. Life is likely to be catalytic and carbon-based. The most easily-conceived scenarios for the existence of extraterrestrial life posit the presence of a prebiotic mix similar to that which existed on this planet. Evolutionary paths different from those that occurred on this planet may have led to the generation of slightly different building blocks and polymers. Life Detection methods should be potentially capable of recognizing the products of these variant paths, as well as proven to be capable in recognizing the various known forms of life on Earth. An overall strategy for life detection is shown in Figure WDP-3 on the next page.

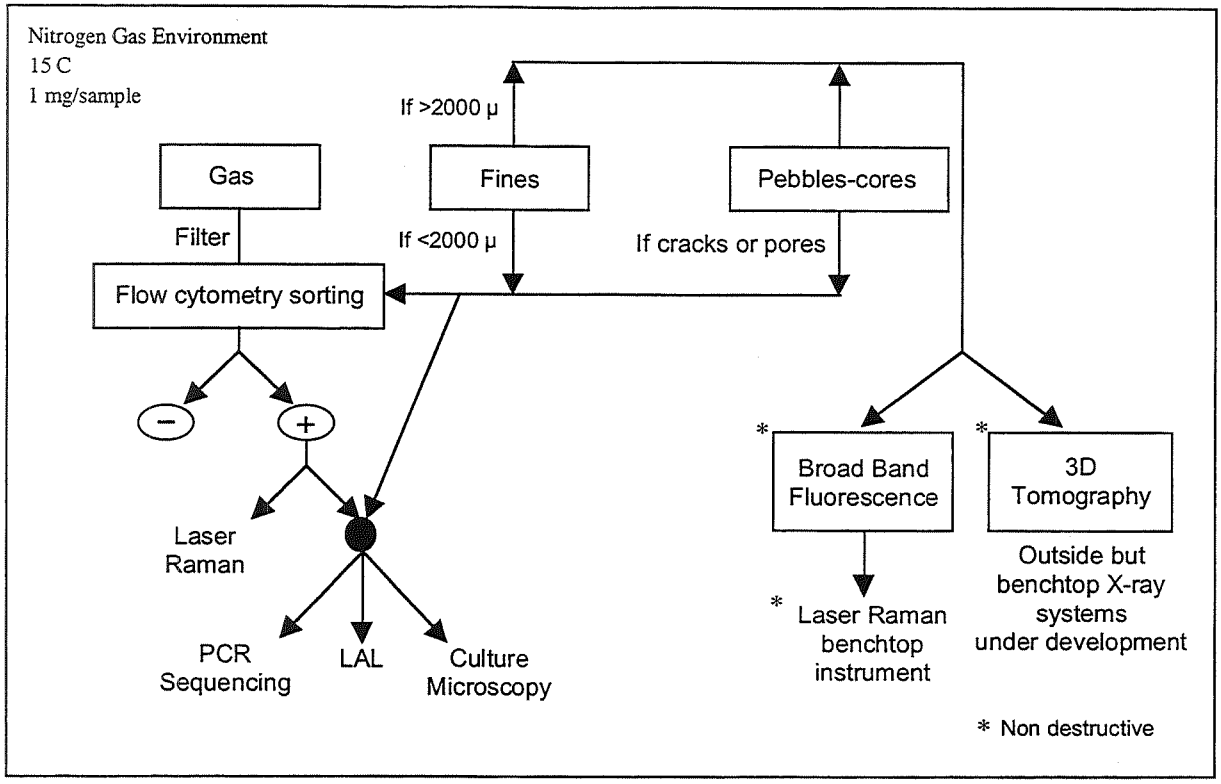

Figure WDP-3. Life Detection Process Flowchart.

Table WDP-3 on the next page, lists what could be considered 'universal' properties of life. Many of these properties are directly measurable, although some of them, such as replication or evolution, can, in all likelihood, only be inferred. Evidence for only a subset of these properties in an extraterrestrial specimen might constitute a sign of life (e.g., evidence for a self-sustaining catalytic system); however, it is the combination and presence of all of these properties that define life as we know it.

Biosignatures: Signatures and signs of life may be defined through different prisms, perspectives, and methods. Broadly-defined signatures (see below) offer the greatest opportunities for detecting life that is unfamiliar to us in its detail; however, broad signatures also carry the greatest chance for misleading or false-positive findings. In general, the greater the number of independently-defined signatures that are detected, the greater the spatial co-localization of these signatures, and the greater the number of separate but clustered co-localized signatures, the more strong is the evidence for life. As a simple example, self-sustaining catalytic processes should create a localized overabundance of a discrete set of related compounds. Conceptual and analytical approaches must recognize that if evidence of extraterrestrial life is found, it is most likely to reflect life that is now extinct or inactive. Useful biosignatures may exist in a variety of types:

- *Morphological:* As we know them, all forms of life are defined by a boundary (e.g., a wall) that delineates them from the surrounding environment. This "spatial-physical incongruity" often contains patterns, complexity and recognizable features (e.g., size, shape, structure, morphological indicators of replication or specialized features such as attachment and motility structures, septae, etc.).

- *Structural Chemistry:* Life can be defined by basic chemical features, such as organic or complex carbon, or by higher-order features, such as polymers, membranes, attachment and motility structures. Methods need to be improved for characterization of complex polymers, and criteria developed for interpreting the patterns associated with complex carbon. We are even less well-informed about the possible structural complexity that can be incorporated into silica and silica-carbon polymers.

- *Metabolism and Bioenergetics:* The waste products that are released, and the energy expended by all forms of life as we know them, can be detected with physical and chemical methods. More work is needed to assess the range of metabolic mechanisms and products that occur on Earth, as well as those that occur in the absence of carbon. Some products are created through specific enzyme catalyzed reactions, such as the reduction of nitrogen that can occur from inorganic reactions. Other products are predicted to result from reactions in the absence of protein-enzymes, such as those involved in energy and CO_2 reduction.

Table WDP-3: Universal Properties of Life (?)

- Life is catalytic
  - + There should be significant deviations from what is predicted by chemical kinetics
  - + Life consumes energy
  - + Life creates waste products
  - + Life is exothermic
  - + Life modifies its environment
  - + Life uses thermodynamic disequilibria to build and maintain other thermodynamic disequilibria (in open systems or within a "wall")

- Life is genetic
  - + There will be some system for storing and propagating information
  - + There will be molecular distributions with significant capacity for complexity

- Life replicates and evolves
  - + There will be evidence for replication of structures and complexity
  - + There will be evidence (structural and chemical) for evolution of form and function

- *Biosynthetic Mechanisms:* All life has mechanisms to synthesize structural, metabolic and replicative macromolecules. Carbon-based life utilizes protein-enzymes and to a limited extent, ribozymes (catalytic RNA). The synthesis of macromolecules involves a sequence of reactions that depends on the availability of basic organic components such as amino acids (for protein synthesis). In taking a broader view, we must consider the possibility of biosynthetic mechanisms and pathways that are catalyzed by inorganic metals and minerals, or are that are dependent on physical gradients (temperature, pH, Eh, magnetism), catalytic mineral surfaces, and various energy sources (UV and other forms of radiation and light).

- *Isotopic Signatures:* All forms of life with which we are familiar fractionate various elements; thus, fractionation patterns can be indicative of life. Organisms that express different metabolic capabilities display distinctive patterns in the fractionation of carbon, nitrogen and sulfur. This might be particularly important in assessing the possible origins of organic compounds and various volatiles such as methane, carbon dioxide and carbon monoxide, if detected on Mars. While one cannot assume that extraterrestrial life will fractionate elements in the same manner as terrestrial life, it is reasonable to assume that local patterns of fractionation within or at sites of life-forms will vary from those measured in the surrounding environment.

  Some isotopes, such as those for oxygen (detected in carbon dioxide and phosphate), can be indicators of environmental temperature. There is promising new technology for measuring carbon isotope fractionation patterns in single organic molecules and fractionation patterns in transition metals. The latter may be very important in identifying a biological source for various minerals such as magnetite.

- *Geochemical Signatures:* This family of signatures includes findings such as magnetite, and other minerals out of equilibrium with their normal distribution in the environment, Redfield-like ratios[47] of key elements (e.g., C, H, O, N, P, and S) found in the pigments of terrestrial life, such as those known to be associated with photosynthesis, and other inorganic chemical anomalies (e.g., based on iron, sulfur, etc.). When specific biologically important elements are limited in the environment, there will be higher concentrations associated with life-forms or colonies of life-forms. Usually, the limiting element in the environment will limit the extent of growth and productivity of organisms (known as Liebig's Law of the Minimum). Some key elements that are limited in terrestrial environments include iron and molybdenum (essential for nitrogen cycle reactions), and tungsten (essential for specific enzymes in hyperthermophilic archaea).

One factor that may complicate Life Detection efforts is the difficulty in detecting or interpreting many of these signatures if the life-forms are inactive, or have been for long periods of time (e.g., hibernation or quiescence), or have become fossilized. One of the large challenges in Life Detection is a more complete understanding of the stability of various biosignatures over time and their dependence on continued metabolic activity.

47. The 'Redfield Ratio' describes the ratio of carbon to nitrogen to phosphorous (C:N:P) found in marine organisms.

There are three possible outcomes of the Life Detection procedures:

1. Failure to detect any of the biosignatures described above, and absence of any carbon or complex carbon in representative samples. This result would lead to proposals for downgrading of containment level for controlled distribution.
2. Clear and overwhelming evidence of living organisms that appear to be of non-terrestrial origin. This finding would likely mandate containment of all samples for an indefinite period of time. Biological experimentation and biohazard assessment would be given highest priority. It must be emphasized that the most likely source of life detected in the martian specimens is terrestrial contamination (just prior to, or following the space mission).
3. The third and most likely scenario lies between these extremes, and would be exemplified by situations in which complex carbon-containing compounds are detected in the sample, but without other evidence of life.

Principles: General principles to follow in searching for life are shown in Table WDP-4. Methods can be divided into those that facilitate a wide survey of a representative portion of different sample types, and those can facilitate a more focussed but high-resolution examination of areas of interest. Survey methods are less destructive of samples, and include microscopy, broad band fluorescence, surface scanning and chemistry, tomography, and isotope release experiments. These methods seek structural and basic chemical signatures, and local inhomogeneities. Higher resolution methods are generally more destructive, and include mass spectroscopic methods, combustion, isotope analysis, and electron microprobe procedures for elemental mapping. These methods seek to characterize inhomogeneities, and more complex structures. An estimate of the sample requirement for the survey, less-destructive methods is 200 milligrams.

General Principles Guiding the Search for Life:

- Begin with a broad survey of a portion of different sample types for more general features suggestive of life, then turn to a higher resolution examination of sites with suggestive features for more complete characterization
- Emphasize structural signatures of life and other inhomogeneities that can be easily detected as a first order task
- Emphasize less destructive methods in the early stages of investigation, since they can guide the use of more definitive but destructive methods
- Start with samples which are the least likely to contain life (e.g., surface fines); if negative, use these as blanks and controls for spiking experiments
- Recognition of life will require the coincidence of multiple independent signatures
- Inactive or "past" life will be treated as potentially active life
- Generalize a carbon-centered methodology to other chemical species
- Use an iterative approach for the Life Detection protocol
- Invest significant time to the design of controls and blanks, as early in protocol development as possible

Some indicators, either structural and/or chemical, which may indicate "past" or inactive life should be treated as potential indicators of active life. One potentially useful strategy for detecting active life-forms is based on replicate measurements over time. Repeated analyses for any of the biosignatures described above may reveal changes in the sample due to metabolic activity. The search for significant changes in these signatures offers an important potential source of information, and does not require a thorough understanding of the signature. The probability of life based on another chemical species than carbon is rare, but cannot be eliminated. With this in mind, carbon centered methodologies and approaches which dominate our present thinking need to be generalized to other chemical species whenever possible. An iterative general approach is recommended for the Life Detection tests, with results obtained by one method or analysis being used to specify and direct any such subsequent methods or analyses.

Analytical Methods: Because deep and surface mineral particles are common micro-environments for microbial life on Earth, the chemical analysis of Mars samples at a micrometer scale can yield information about the presence of active or fossil life on Mars. Raman, IR, and fluorescence micro-spectroscopy are valuable tools to perform non-destructive analysis of mineral matrices and surface compounds.

- *Microscopy:* As part of the preliminary examination of returned samples, light microscopy of fines as well as surfaces of pebbles or rock should be used to look for obvious signs of cellular structure and mineral deposits associated with microbial life.
- *Analysis of Gases in Head Space:* Analysis of a pristine atmospheric sample should be compared to a similar analysis of gas occupying the head space above collected soil and rock samples. Differences may be due to chemical interaction of the gas with samples, or may be signs of metabolic activity within the specimens.
- *Laser Desorption Mass Spectroscopy and Laser Raman:* Laser desorption mass spectroscopy (LD/MS) and Laser Raman analysis are rapid, non-destructive methods for detecting low levels of organic matter in geological specimens. They have been successfully used to analyze PAHs in meteorite and interplanetary dust particles. Minimal sample preparation is required and small particles as well as fresh fracture surfaces of larger specimens can be analyzed. In LD/MS, a 10-40 micron diameter spot is positioned on the specimen, organic species are thermally desorbed from the outer few microns of the specimen, they are photo-ionized and directed into a time-of-flight mass spectrometer. Continuing developments offer the prospect of high selectivity in detection of specific classes of organic compounds, (e.g., amino acids). Automated scanning technology will be critical for application of these techniques to the maximum amount of sample. The techniques are limited to surface analysis.
- *3D Tomography:* Given the present state of the art, 3D tomography would require transport of a specimen outside of maximum containment facilities to a synchrotron; however, the specimen can remain in a sealed container, under the equivalent of PPL-α containment conditions.
- *Carbon Analysis:* High priority should be given to quantitative analysis of carbon, especially organic carbon. Techniques having the greatest sensitivity should be applied, including progressive heating/oxidation, coupled to GC/MS. It is anticipated that multiple samples and

sites with suspicious findings from survey methods will be analyzed to detect and characterize localized organic or inorganic carbon.

- *Flow Cytometry:* An aliquot of the aqueous slurry will be subjected to flow cytometry. Flow cytometry will be used to analyze single particles in the range of 2 to 100 microns in diameter at rates of tens to hundreds of thousands of particles per second. Based on initial, non-destructive characterization of laser light scatter and auto-fluorescence, particles will be re-analyzed, with or without staining with fluorochromes specific for DNA, proteins or functional viability assays. During subsequent analysis, at least four pre-selected sub-populations can be sorted from each sample for further analysis by other techniques. Positive fractions can be sorted and directed toward further chemical and biochemical testing.

Extraction of Representative Sample: It is anticipated that sample material will differ in size and composition. A representative aliquot of approximately 1 gram should be subjected to extraction for further destructive tests. The initial extract will use ultra-clean water. Mechanical disruption may be necessary, but should be kept to a minimum so as not to damage cellular structures or potentially viable cells. A fraction of this aqueous slurry should be designated for organic solvent extraction.

Cultivation: Elaborate forward-contamination controls will be used on the mission, however it is still possible that viable terrestrial microbes may be detected in returned Mars samples (either from contamination on the original spacecraft, the sample container that made a round-trip, or through sample handling contamination). To rule out possible terrestrial microbial contamination, an aliquot of the sample should be subjected to the standard microbiological examination currently used for Planetary Protection, as well as other routine methods for detecting and identifying terrestrial organisms. Culture conditions that would be compatible with martian micro-environments are not well-understood, yet attempts should be made to create such, and propagate life-forms. The composition of gases in the martian atmosphere, both present and ancient, should be replicated, especially with CO_2 as a carbon source. Given the extremely dry conditions on Mars presently, the degree of sample hydration should be varied. The range may fluctuate from partially hydrated specimens to totally aqueous conditions. Energy sources should include light for any possible photosynthetic organisms and pairs of electron donors and acceptors for chemosynthetic organisms. Mineralogical information from samples should be integrated into the decisions in media formulations. Likewise, any organic compounds detected in the samples should be considered as carbon sources.

Cultures will be monitored by simple microscopy as well as through multiple sequential analyses by GC/MS, LC/MS, micro-calorimetry, nucleic acid amplification and other methods.

Distinguishing Earth-based from Mars-based Life: If viable cells are found in the samples, it will be important to first rule out the possibility of terrestrial microbial contamination. Cells will be subjected to phenotypic and genotypic analyses. Searches against databases with known terrestrial organisms will quickly identify contaminants. In a similar fashion, the most likely source for familiar complex polymers such as nucleic acids is from terrestrial contamination. Amplification techniques such as the polymerase chain reaction (with broad range primers directed against targets such as rDNA, and with random oligomers) and subsequent sequencing methods offer a sensitive and rapid means for detecting and characterizing DNA and RNA (as a marker for terrestrial contamination), and should be applied to the outbound spacecraft, container surfaces before and after return, as well as the samples

themselves. Other assays, such as the Limulus Amoebocyte Lysate (LAL) assay, may assist in detecting extremely small amounts of terrestrial contamination, but are less specific. One should keep in mind that detection of terrestrial contamination in a specimen does not exclude the possibility that the same specimen also contains martian life.

Considerations Concerning Controls and Blanks:

- Prior to departure, the spacecraft and specimen containers should be examined, and samples should be archived. Witness plates should be employed.
- Strong consideration should be given to the return of a sample of martian atmosphere in a separate, but identical container. If collected and stored under increased pressure, extra aliquots of atmosphere could be used for replication of martian conditions in other experiments after specimen return.
- Early determination of negative findings for life in low-likelihood martian samples may allow these samples to be used as negative controls.
- Because negative results are expected in many of the Life Detection procedures, determinations of assay sensitivity using known specimens of terrestrial life would aid in the interpretation of these negative results.
- Methods should be validated and evaluated using a wide variety of terrestrial life-forms.
- Simulants of martian samples and conditions should be refined for protocol development prior to sample return. Particular attention should be given to the probability of highly-oxidizing sample surfaces.
- Exposure of the sample surface to PPL-α conditions will inevitably lead to deposition of particulate matter from the surrounding environment. The features of this process should be characterized prior to specimen return.
- Finally, an effort should be made to ask questions that yield interpretable answers, (e.g., answers for which a statistical assessment of confidence can be performed).

Life As We Don't Know It: Assumptions about the nature of life are listed in Table 3. The possibilities of dealing with "life as we don't know it" were also considered, including a composition devoid or organic carbon, the unconventional reliance on "non-biological" elements such as Si, Fe, and Al, structures less than 100 nanometers in diameter, and a composition based on organic monomers. It is difficult to evaluate the probability of encountering forms of life with these features.

Discussions of the possibility of non-carbon based life has had a rich history, especially in the realm of science fiction.[48] Life based on organic monomers has recently been proposed as a model for the 'metabolism-first' scenario for the origin of life.[49] According to this model, a set of self-sustained chemical reactions might be considered 'living' if metabolism is considered to be more important than replication as a fundamental basis of life (see discussion and Table WDP-3 above). Some of these unlikely scenarios might require alternative laboratory conditions for proper study (e.g., the use of inert gases).

48. H.G. Wells, writing in the Pall Mall Gazette in 1894, scolded scientists for thinking of only carbon-based life: "It is narrow materialism that would restrict sentient existence to one series of chemical compounds – and the conception of living creatures with bodies made up of the heavier metallic elements and living in an atmosphere of gaseous sulfur is no means so incredible as it may, at first sight, appear."

49. Wächtershäuser, G., *Science* 289:1307-1308 (2000).

Existing theories of the origin of life on Earth suggest that life will arise as a consequence of chemical and physical principles anywhere prebiotic carbon compounds accumulate in suitable environments (e.g., water, temperature, etc.) in sufficient amounts for sufficient time. Although the precise process for life's origins on the Earth is not known, it is perceived to have been a progression in complexity beginning from an original prebiotic mixture, at some stage involving RNA catalysis, and probably at later stages catalysis by peptides and proteins, ultimately culminating with the first simple organisms that had a metabolism, the ability to replicate and the capability of preserving useful information during the replication process. The most likely scenario that we can conceive for the development of life on Mars is by a similar process, which if stochastic, may have deviated from our own terrestrial process, resulting in different fundamental amino acids or nucleotides used, types of lipids, chirality, etc. The primary indicator of past or present life of this type would be the finding of unusual macromolecular assemblages (e.g., peptides or oligonucleotides with nonstandard amino acids, nonstandard bases, non-standard linkages). If deviation occurred only later in the process, then we might find Earth-like complex structures, such as recognizable ribosomal RNAs.

Sample and Time Requirements: It is estimated that approximately 3 grams of sample will be required to conduct the proposed preliminary Life Detection tests on returned martian sample materials.[50] As methods mature and new approaches become available, these sample requirements may change. Estimations of the time needed for Life Detection are difficult to provide. Survey methods can be completed within weeks-to-months, in some cases. However, any positive or suspicious findings may impose additional time requirements, depending on the strength of the findings and the follow-up methods required for further assessment. Enrichment culture experiments, for example, will likely extend for many months.

Need for New Technology, Methods, and Database Development:

- Miniaturization of many chemical/physical analyses
- Sample registry, for re-interrogating precisely defined sites
- Micro-calorimetry
- Database development
- Software for "multiple sequential analysis" search logic
- Effect of Mars versus inert atmosphere on proposed methods
- Cleaning/cleanroom technology
- Validation of controls
- 3-dimensional nano-scale physical mapping of specimens
- Characterization of complex compounds based on Si, Al, Fe
- More complete inventory of life on Earth, using molecular methods

Biohazard Testing

The Biohazard testing regime was designed to determine if samples from Mars pose any threat to terrestrial organisms or ecosystems, whether or not the samples are found to contain life-forms or non-replicative hazards. In designing the Working Draft Protocol, it was recognized that potential

50. Estimates for sample amounts are based on what is necessary to do the tests outlined in the Draft Protocol, however, actual amounts may depend on definitions of "representative samples" made at the time samples are returned.

hazards could take one or more of a multitude of forms (e.g., toxic, mutagenic, life-cycle altering, hazardous through genetic recombination, disruptive to ecosystems, capable of biasing phenotypes or even behavior). Thus, the spectrum of tests selected for assessing the nature of the hazard(s) is deliberately diverse.

The output of the Biohazard testing process will be used in combination with Life Detection and Physical/Chemical tests to determine what level of containment, if any, will be required for the samples. In practical terms, the final protocol should allow a determination of whether the samples contain any biohazards and whether or not to distribute sub-samples – with a high degree of confidence and a clear definition of the conditions of release. Determination about sample release from containment and will be made with careful consideration of applicable regulatory requirements and will provide reasonable assurance that the distribution of samples will not put humans or other terrestrial organisms at risk.

The proposed tests and procedures for the Biohazard testing regime reflect current state of knowledge and practices. It is anticipated that the Working Draft Protocol will evolve both in content and implementation as a result of new or improved methodologies or expanded state of knowledge prior to sample return, and in response to real-time information about sample materials learned during implementation of the various processes at the Mars receiving facility.

Biohazard Defined: In general terms, hazards of concern to biological systems are those substances (materials or entities of biological origin or not, replicating or able to be amplified[51] by a biological system or not), capable of producing an adverse effect or significant alteration[52] on a biological system at the level of individual organisms or ecosystems. In considering returned martian samples, a distinction has been made between replicating and non-replicating hazards. For the purpose of this Working Draft Protocol, a biohazard is defined as a hazard that can replicate or be amplified by a biological system. In practical terms, replication is a key distinction between a biohazard (i.e., replicating and potentially contagious) and a simple toxin or hazard (i.e., a non-replicating hazard that can be diluted down below a toxic concentration). Only replicating entities or entities that are able to be amplified by a biological system could pose a potential widespread threat. While toxic and other hazardous materials are of concern, they represent a potential hazard only to staff and scientists who may be exposed to them.

If the distinction between a biohazard and a hazard can be made, the level of containment and procedure for distribution of the samples can be appropriately defined. The existence of either biohazards which are self-replicating or able to be amplified by another biological system or toxic hazards would require further study and characterization of the nature of the hazard (e.g., strong chemical oxidizer, radioactive, replicating life-form, etc.) so that appropriate subsequent containment and/or handling procedures can be determined and stipulated to avoid potential biological impacts during future research. However, the existence of either a biohazard or a hazard in the samples in no way precludes subsequent scientific analyses.

51. In this context, biohazards are not limited to 'living' entities and may include biohazards such as viruses that are not living or self-replicating *per se*.

52. In the context of potentially biohazardous extraterrestrial entities, "adverse effects" includes to any significant alteration on a biological system and is not limited to adverse effects that are immediately or acutely toxic.

Assumptions About Containment: Containment at the SRF will be designed to cover a range of conditions while maintaining martian materials under appropriately strict biocontainment. It is important to understand the various containment types at the sample return facility and the anticipated containment needs during Biohazard testing. Life Detection tests and Physical/Chemical tests will seek to characterize the sample materials and determine if evidence for "life" can be found while testing under conditions that are both Mars-like (e.g., pristine environment) and Earth-like. In contrast, Biohazard tests are only designed to determine the effect of martian samples on terrestrial life-forms under Earth-like conditions (an important distinction). Thus containment requirements for execution of the Biohazard testing will not require the stringent clean room conditions associated with preliminary physical/chemical tests, certain Life Detection studies, and 'banking' or curation. The appropriate initial containment level for the Biohazard testing regime is thus anticipated to be PPL-γ, which translates to the maximum BSL-4 biocontainment, but with less strict cleanliness restrictions.

All Biohazard testing will be conducted under strict containment at the primary receiving facility or other similarly secure maximum containment facility. Since neither all the necessary scientific experts nor the high-end scientific instrumentation they require are located at a single facility, there may be a need to allow samples to be distributed for study/curation at facilities other than the initial receiving laboratory. Some tests may be done at locations other than the primary receiving and maximum containment facility as long as maximum containment and security of the sample are maintained (i.e., the sample must be kept completely isolated within multiple containers that are appropriately nested, sealed, and intact). The rationale for being able to test un-sterilized sample materials outside of the primary containment facility is dependent on the availability of adequate procedures for containing and transporting the samples, for sterilizing or cleaning the outside of the sample container, and for returning the sample to the containment facility after non-invasive or non-destructive analyses (e.g., synchrotron analyses). Mobile containers certified at the appropriate PPL level (as distinct from traditional BSL transportation requirements) should be used for transport of samples between facilities.

The unknown nature of any possible biohazard in returned martian samples demands, at least initially, the most stringent containment presently afforded to the most hazardous biological entities known on Earth. If sufficient data are gathered to rule out concerns about human virulence and infection, a decision could later be made to allow subsequent work at a lower containment level during tests investigating possible environmental effects. The Biohazard testing process is designed to allow for gradual decontainment or adjustment to less stringent containment levels if justified upon review of accumulated data about the sample materials during implementation of the final protocol.

If the initial Life Detection and Biohazard tests are all negative, it would be appropriate to conduct subsequent tests under less strict containment conditions once sample materials have been shown to be non-biohazardous. In particular, additional geophysical testing can be done at a reduced level of containment as well as selected biological tests associated with the biohazard analysis. A lower level of containment would potentially enhance sample access within the scientific community while still providing adequate biosafety conditions under existing biosafety guidelines and regulations.

Elements for a Biohazard Testing Regime: Considering that Biohazard testing should yield results within a "reasonable time" (e.g., all testing completed within 6 or perhaps 9 months) most tests should be started synchronously and be conducted in parallel. However, the influence of preliminary sample

examination and work on Life Detection may lead researchers to proceed with some tests before others. Gradual "de-containment" strategies require identifying biohazards to people before identifying biohazards to the environment.

The general strategy that emerged was to prioritize the types of assays in terms of the impact of potential pathogenicity on distribution to other laboratories. If a possible human pathogen were detected, the strictest of handling protocols would remain in place. If a pathogen that was specific to a particular host were detected (not likely), less stringent handling methods may be possible (for example, many virulent animal and plant pathogens are handled safely and routinely at lower containment levels than human pathogens). If a non-replicative toxic agent (e.g., toxin) were discovered, containment issues would be less restrictive and definable using dose-response characteristics and the nature of the toxicity.

Prior to conducting biohazard tests, decisions will be needed on what model systems should be selected to make up the specific assays. The working criteria for choosing the models are given below:

- The models should be relevant to a probable hazard scenario, deliberately avoiding models that would only be sensitive to an improbable danger (i.e., very unlikely event, very artificial route, extreme doses, rare species confined to remote niches, etc.) as such models would be of little relevance to initial Biohazard testing with Mars samples. The emphasis will thus be placed on modeling of biological systems likely to be in contact with samples (i.e., workers, their microbial flora, their pets, insects, life-forms common to the surrounding of sites of future experimentation with the samples), via probable routes of exposure (i.e., aerosol, etc.), at probable (low) doses.
- Subsequent models should be relevant to systems of ecological and/or economic interest.
- All models should ideally be sensitive, meaningful and easy to interpret. Equivocal answers can only prolong time to potential sample release and can use up samples unnecessarily.
- All models should ideally be robust. Samples are likely to contain complex minerals, oxidative agents and other elements that should not interfere with its function.
- All models should ideally be well documented. Observations and analyses should identify known behavior of the biological system in the model. Preferably, its genome should be fully sequenced, and extrapolation to other species/situations should have been evaluated.
- All models should ideally provide answers in a reasonably short time.
- All models should be ideally compatible with handling within the SRF, under containment.

Sequence of Tests: Table WDP-5 is an outline of a possible pathway of experiments for Biohazard testing with estimates of sample usage for each set of experiments. A flow chart for this pathway of tests is shown in Figure WDP-4. The text below provides a narrative explanation of that figure.

Since fines can be considered 'homogeneous' and can be sub-sampled as a single category in a statistically relevant way, Biohazard testing should begin with fines. Whether and when other materials should undergo the full array of Biohazard testing will be based on the results of initial P/C screening and processing.

Initial Testing: The initial biohazards tests, which have a specific focus on determining adverse effects on humans, will be done in PPL-γ (Containment: BSL-4, Environment: Normal terrestrial). Toxic effects on cultured cells and microorganisms should be anticipated due to the chemical (mineral) composition of the Mars samples. Appropriate controls (terrestrial or meteoritic) must be run and interpreted. It is assumed that toxic effects, if any, should diminish rapidly in sub-culturing ('passaging') experiments, since a replicating agent or one able to be amplified would not be involved in a toxic response *per se*.

It was recommended that specific cell and tissue systems be used for Biohazard testing. It is envisioned that a large amount of the cell culture work will be done robotically using existing or new technologies.

The following specific initial tests are posited *[Race et al., 2001a]* to be included in the Working Draft Protocol, should it be carried out today:

- Human cell lines and primary cell cultures, with particular emphasis on epithelial cells (e.g., skin, lung, gut). All cells will be observed for abnormal growth (e.g., cytopathic effect, morphological changes, genetic response to stress, integration into host genome, co-growth [mycoplasma-like], and mutation rates). Cells can be checked for transformation (growth on soft agar). Both supernatant and homogenized cell pellets should be passaged, typically twice each week for 3 months. Other replicate cultures must be observed for 1-2 weeks to look for delayed effects.
- Mouse cells should also be tested, with "culture-adapted" material being injected into mice. Three mouse systems should be employed: wild-type, SCID, and SCID-Hu.
- Microbial systems to be tested should include: *Chlamydomonas* (stress response), *S. aureus*, yeast, and *E. coli*. In addition, microorganisms that grow in high salinity should also be considered.

Subsequent Testing and Possible De-containment: If the initial Biohazard tests (above) and Life Detection tests are all negative, it would be appropriate to conduct subsequent tests under less strict containment conditions (e.g., PPL-δ). In particular, additional geophysical testing can be done at a reduced level of containment as well as some additional Biohazard tests using the following models:

- Secondary mammalian cell culture systems.
- Plant cell systems (*Arabidopsis*) and whole-plant growth experiments.
- Additional microbes (e.g., nanobacteria, cyanobacteria, thermophiles, anaerobes, gram-positive bacteria) and microbial systems (e.g., various temperature ranges, pH ranges, salinity).
- Other species: *Drosophila melanogaster* (e.g., wingless mutants), worms (*C. elegans*), and amphibian and bird eggs. Horizontal and vertical transmission studies should be done. (All animal species should be observed for behavior change, toxic and teratogenic effects, and pathological changes.)

Additional experiments can be done using a variety of techniques to test for biologically active compounds, micro-arrays (for proteins), etc.

Test Type	Procedures/Questions	Sample Usage and Time Required
Verification that any potential organisms do not attack biocontainment materials (e.g., Silastic™, rubber, etc.).	Do samples affect test coupons of containment materials at various humidity levels and temperatures?	Sample expended: 1 gram Time: 1 - 3 months?
Input from Life Detection Procedures (discussed separately): • If life detected, this would radically change/focus the approach to Biohazard Testing by providing focus in terms of conditions for replication, agents that can kill the organism(s), etc. • If no life is detected, still run subsequent tests for toxicity and biohazard.	• Carbon? • carbon-carbon bonds? • Complex carbon compounds (indicative of metabolic processes)? • Skeletal remains or fossilized remnants? • Indication of live organisms (organelles, membranes, structures on microscopic evaluation)? • Life-like structures? • Living agent (replicates in environment, with co-agent/host, in terrestrial cells)? • Mutual/commensal/parasitic relationship? • Kills cells or organisms? • Kills complex multicellular organisms? • Kills everything?	Sample expended: TBD Time: TBD
Multi-species infectivity, pathogenicity, toxicity testing. • Look at broad host ranges (assuming that any pathogens would not be too host-specific) with well-known and standardized model systems. • Use small organisms in small volumes, allowing for maximum sample conservation. • Initial work all done at BSL-4 biological containment level.	Sample preparation (rough cut): • Crush larger clumps/rocks but do not pulverize particulates. • Filter? • Mix into sterile water. • Chelate heavy metals? • pH buffer? • Use serum for some samples? Heavily irradiate sterilized control samples w/ ^{60}CO. Introduce appropriate amount of sample (10 - 100 milligram for statistical relevance) to culture of unicellular organism and cell lines. Inoculate whole organisms (animals to model humans) with primary (not passaged) material. Monitor: • cell proliferation, • cell morphology, • deferential analyses of biochemicals and gene expression • comparative genomics (any inserted genes in host?) • reporter assays (?) • etc.	Sample expended: Three trials plus sterilized control per organism, assuming 100 mg per sample = 1.6 grams. Time: ~ 6 months to allow for passage times.
Negative results with multi species tests may lead to downgrading to PPL-δ.	The following tests/criteria are proposed: • First passage from infectivity analysis (+ or –), but second and subsequent passages all neg. • DNA damage assays (mutagenesis: Ames test, strand break analysis). • Environmental damage. • Whole plant inoculations. • Diversity of growth conditions extant on Earth (extremophiles, etc.) and other media. Monitor: cell viability, expression of toxic response genes. Neg results in these tests may allow a decision to downgrade to a lower containment level or release.	Sample expended: ~10 - 20 grams (very rough estimate). Time: ~6 months to allow for passage times. Note: There was consensus on the 'first round' (infectivity), but it was also clear that the containment level determination issues need considerably more analysis and study.
		Total = 15-25 grams

Table WDP-5. An outline of a possible pathway of experiments for Biohazard testing.

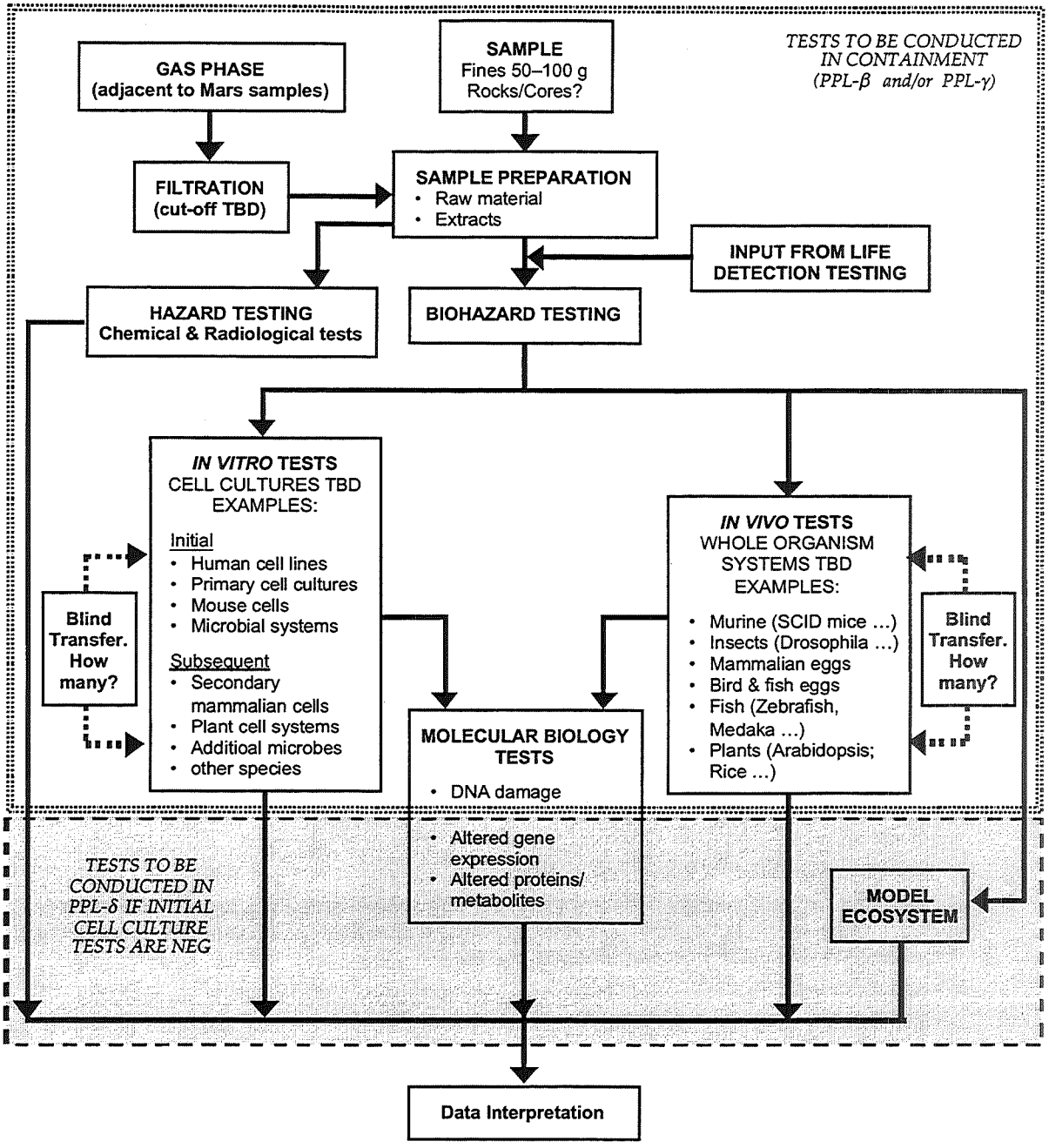

Figure WDP-4: Proposed Flow Chart for Biohazard testing after verification of containment materials integrity.

Verification of Containment Materials Integrity: A set of preliminary tests is required, relating to materials used in containment equipment. As a starting point to Biohazard testing, it is important to verify that sample materials or potential organisms growing from them do not attack rubber, Silastic™, and other bio-containment materials.

For example, one might take ten 10-milligram samples for each seal/containment material (e.g., latex, Silastic™, Plexiglas™, cyanoacrylate, epoxy, etc.). 'Coupons' (i.e., small, regular samples) of each material would be incubated at a few different humidity levels, bounding those actually to be used for sample curation, and including liquid water. Test vessels for these experiments (i.e., primary containment) should be extremely non-reactive, such as refractory metals (e.g., titanium). For this example, if ten materials are tested, a total of one gram (or less) of martian sample would be expended.

At regular intervals (over weeks to months), the sample coupons would be monitored for degradation using optical methods, mechanical tests, and chemical analyses. 'Failure' criteria would be defined in terms of parameters that would compromise containment, such as outright consumption, pitting/erosion, pinhole formation, substantial changes in bulk chemical or mechanical properties, etc. The results would be used to provide a high level of confidence that the samples could be kept in storage vessels made of the tested materials without risk of inadvertent release.

Preliminary Biohazard Tests: The set of preliminary Biohazard tests include:

- *Direct culture:* Part of the Life Detection testing process; any cultured organism which can not be clearly identified as terrestrial will be subjected to a biohazard study.
- *Cellular and 'small' models:* Unicellular organisms, or very small animals can be used with limited amount of sample, ~10-1000 micrograms per test. These tests would be based on simply exposing the organisms to the sample and using some form of signal readout, such as gene expression. Should the organisms or cells be chosen or developed *today* (which is *not* recommended), they would probably include:
  - > Wild type, mutant and recombinant yeast bearing special sensitivity to hazardous material (e.g., radiation mutants, GFP and BFP (green and blue fluorescent proteins, respectively) recombinants to test for recombinogenicity, etc.);
  - > Human cell lines as sensitive to pathogens as standard cell lines which are used for Biohazard testing (e.g., A human equivalent to vero E6 cells), as sensitive as BHK-cells to mutagens, etc.);
  - > Bacteria found associated with people (e.g., *E. coli, Staphyloccocus, Bacteroides*, etc.);
  - > Bacteria found in niches likely to be similar to martian underground ecosystem (i.e., probably cold and possibly oxidizing, low-oxygen and with high radiation levels);
  - > Relevant algal/planktonic unicellular organisms;
  - > Mammalian (e.g., mouse) egg before re-implantation;
  - > Fish eggs (e.g., Zebrafish, Medaka, etc.) For testing effects on development; *Neurospora crassa;*
  - > Cells and seeds from Arabidopsis and rice;
  - > Complete *C. Elegans; and,*
  - > Complete *Drosophila melanogaster* (likely a flightless variant).

- *Mutagenesis Assays:* One possible approach is mutagenesis assays that look at genetic changes over several (rapid) reproductive cycles. Typically, this is done with bacteria (e.g., with the Ames test for carcinogenicity[53] using *E. coli*). The general consensus was that these tests would be problematic in that mutagenesis results tend to be oversensitive and controls would be difficult to realize. A related assay type is terratogenicity, but these require breeding animals, and thus can be more lengthy (for some species) than other assay types.

- *Whole organisms:* This approach includes ingestion/inhalation/injection of samples by living organisms with subsequent monitoring of physiologic functions, behavior, gene expression, inflammatory cascade (e.g., cytokine levels), etc. Hosts can include animals, plants, and modified organisms (such as SCID mice, xenograft systems, etc.). Another key aspect of this approach is the ability to evaluate the infectivity of the potential organisms to other organisms via passage.

  The benefits of this approach include: direct measurement of physiologic effects; ability to handle multi-organ interactions in toxicity; inherent inclusion of complex host characteristics (tough to do with cell based and other assays); and, the possibility of detecting infectivity (if hosts are appropriate for replication).

  However, some significant drawbacks exist, including: difficulty in seeing long-term effects; impossible to cover all possible organisms (many terrestrial pathogens are very host-specific); may require large samples; may be confounded by inorganic materials; and, results may depend on mode of introduction of sample to organisms (terrestrial pathogens have specific routes of infection). A major drawback of this approach is that it requires more sample: ~100-5000 micrograms per test. The organisms chosen or developed as of this writing, include:

  > *Arabidopsis* and rice at different stages of development, exposed by direct contact and/or aerosol,
  > Zebrafish and Medaka, exposed to the sample by routes to be determined,
  > Bird eggs (notably embryonated chicken eggs) injected with powdered sample,
  > A variety of types of mice (i.e., germ free/humanized/wild type/mutant/recombinant/ newborn/immunosuppressed/pregnant/reimplanted), exposed to aerosol or *per os* or injected intraperitoneally (i.p.) or intracranially (i.c.) with powdered sample.

- *Molecular and biological tests:*

  > *DNA Damage:* Assessment of DNA damage should include the measurement of mutation frequency, recombination frequency, and the occurrence of DNA strand breaks. Standardized methods are available to carry out each of these measurements, for example, genetic reversion assays for DNA mutation, transposon rearrangement assays for recombination, and terminal transferase assays for strand breaks. Such approaches, focusing on general measures of DNA damage, are likely to be more fruitful than highly specific measurements of DNA damage, such as comparative sequencing or the measurement of a particular type of DNA damage.

53. Ames, B., F. Lee, and W. Durston. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.

> *Altered Gene Expression:* Techniques are available for measuring the relative expression level of almost any gene under various conditions. For purposes of biohazard assessment, however, it would be preferable to narrow the focus to genes that are expressed at a significantly altered level in response to infection or toxic exposure.

> *Altered Levels of Proteins and Metabolites:* Rapid progress is being made in developing chip-based and other methods that allow one to measure the level of particular proteins or metabolites in a biological sample. Within the next five years, driven by the demand of genomics research and drug development, these techniques are likely to become broadly available. It is difficult to make specific recommendations at this time until standardized procedures are established. It is expected, however, that the comparative measurement of proteins and metabolites associated with the biological response to infection or toxic exposure will become part of the biohazard assessment procedure.

- *Ecosystems:* While difficult to define (due to huge numbers of permutations and combinations), multi-organism population testing is important because potential biohazard effects may only manifest within the complex interactions present in ecosystems. Testing for ecosystem disruption seems difficult as few models have been validated. Apart from 'global' parameters (e.g., global metabolism, biochemical profile of solid/liquid/gas phases, etc.) few specific parameters for monitoring have been defined at this point. These tests would be potentially sensitive to both subtle and complex changes, but difficult to define and monitor, and may take long time periods to show effects. This points to a relatively large amount of research and development that will be required to develop comprehensive and effective tests.

Sample Size: Two different approaches were used to estimate the amount of sample required for analysis. The first was based on some sort of pre-sorting of the sample that assumed that 'relevant' biologically interesting sub-samples would be used. With this approach, the crudely estimated sample consumption for Biohazard testing was ten grams; the amount of sample to be used is dictated by:

> the relevance of the dose being modeled,

> the amount the biological model system can physically be dosed with,

> the sample preparation procedure,

> the number of tests to be conducted, and

> the total time Biohazard testing should take.

The second approach did not assume a particular sorting of 'relevant' samples, but instead used simple statistical methods. Using Earth soil as a crude reference, a conservative calculation suggested that 15–25 grams of sample should suffice. These two estimates were quite close, despite very different approaches used to arrive at them.

Ruling out biohazards in one sample will not allow for extrapolation to other samples – it will remain a case-by-case task, at least for a considerable period. This applies even when sub-sampling returned materials. The logical follow-on question is whether or not samples should be 'homogenized' prior to Biohazard testing. Input from geologists indicated that homogenization does not make sense because of loss of information – for example, sedimentary rocks (which may be in the minority) are more likely to harbor signs of life than igneous rocks. In addition, since surface conditions may be toxic to organisms, homogenization with deeper sample components may not be sensible.

In general, small sample sizes will be required to conserve the returned specimens, so biological assays that require small quantities are highly desirable. Examples include cell-based assays (requiring as little as 100 microliters of total fluid volume, making milligram samples potentially adequate) or the use of small organisms, such as *Arabidopsis* and *C. elegans.*

It was noted that the amount of material needed for destructive testing (consumed) in biohazard assessments must be determined in consultation with biostatisticians. Regardless of what type of starting assumptions are made, the statistics of sampling will apply, and confidence in 'hazard exclusion' statements can only be made in the form of "no hazard exists at a concentration greater than X/gram."

Time Needed: The time to conduct Biohazard testing was estimated to be twice the time to conduct the slowest test. It was estimated that 3 months would be too short although most of the results would be acquired within these 90 days. In fact, 4 to 6 months would be preferable. As an example, and in terms concrete to North American researchers, it is estimated that all Biohazard testing necessary to downgrade the samples from BSL-4 to BSL-3, will take approximately 6 months, and another 6 months will be required to downgrade the sample to a lower level of containment or release, as appropriate.

Comments on Controls: Control samples are clearly needed for all of the above experiments. Methods for generating control samples (e.g., dealing with oxidants, iron, etc. – these contaminants could greatly confound bioassays and not be modified by some sterilization methods such as high-level irradiation) must be developed.

Irradiated samples, while somewhat modified, are apparently suitable for much of the geologic investigations of interest. Interestingly, "clean" in terms of geology can mean knowing that certain elements such as gold are present in concentrations in the parts-per-trillion. The important point here is that typical biological containment systems are not designed with such cleanliness (molecular/atomic) in mind. A practical impact of this is that containment/handling equipment and materials should be characterized in terms of trace concentrations of elements that may be irrelevant biologically but damaging to geological and other scientific analyses.

One additional point is that there is a need for pre-launch controls to rule-out terrestrial contamination. Swab samples, etc., from the assembly and launch phases and test facility should be taken two years before sample arrival. This will be a vital piece of the process to establish positive and negative controls. Negative controls can also be generated at the time of analysis by treating samples with DNAses, proteases, etc., to subtract out any terrestrial or Mars biomarkers, so that effects of Mars soil on subsequent assays can be evaluated.

Research and Development Needed: Further efforts need to be undertaken to perfect many steps in the final protocol, including:

- A sub-sampling procedure needs to be developed and validated so as to provide statistical relevance and innate conservatism. This is essential to ensure that the Biohazard testing is capable of determining the safety of the samples. Without an effective representative sub-sampling strategy, testing of the entire sample may be necessary, and untested samples may need to be kept in containment indefinitely.

- Specific models for use in Biohazard tests have to be chosen or developed. Each one of them should be validated with terrestrial mimics of martian soil (possibly with meteoritic minerals from Mars) used as-is, or spiked with known agents to provide a positive control in Biohazard testing.
- Relevant, robust and reproducible methods of sample preparation and sample delivery must be developed to ensure the final protocol can be effectively accomplished.
- The selection of optimal cell and culture systems for use in biohazard and toxicology assays will be critical to avoid potential contamination that could interfere with data interpretation. Prior to implementation of the final protocol, research is needed to select optimum cell and/or molecular assays for Biohazard testing.
- All assay refinements should take into account biohazard containment issues in their design and implementation. Moreover, NASA will likely need to coordinate these refinements and any attendant research developments with the toxicology and infectious disease programs at the NIH, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, and the Centers for Disease Control and Prevention (anticipating forthcoming funding increases to integrate extensive research into infectious diseases and bio-terrorism issues). NASA must also stay abreast of developments in toxico-genomics at the NIH, a new field anticipated to replace conventional toxicology and its antiquated methods over the next 5 years.

Facility Requirements

The size and scope of the facility required to complete the elements of this Working Draft Protocol will depend on whether all protocol functions and activities (e.g., sample receiving and processing, physiochemical characterization, Life Detection studies, and Biohazard testing) will be conducted at a single SRF or some elements will be distributed to secondary labs beyond the SRF. Based on experience following receipt of lunar samples, regardless of whether some components are distributed to multiple sites, the primary SRF should be designed to be expandable and to allow great flexibility in switching functions as needed. In particular, the SRF should be able to support primary investigator-driven research, if needed. This single primary facility (or at most duplicated facility) should be designed to allow continuous and long-term operation in addition to its primary goal of receiving the Mars samples and completing this Protocol. There also should be a backup facility at PPL-α to contain a subset of the initial samples for banking purposes.

The various protocol elements and appropriate levels of containment are depicted in Figure WDP-5 on the next page shows a sample processing schematic with containment requirements by test category. From a planetary protection consideration, these functions can be performed at any facility that meets the containment requirements. Similarly, no specific test or instrument is precluded from use during the completion of the final protocol if that test or measurement can be done or placed in containment. Regardless of how the final protocol functions are distributed, all ancillary facilities must meet the same containment guidelines and standard operating procedures (for items such as personnel monitoring, security assessment, chain of custody tracking for samples, etc.).

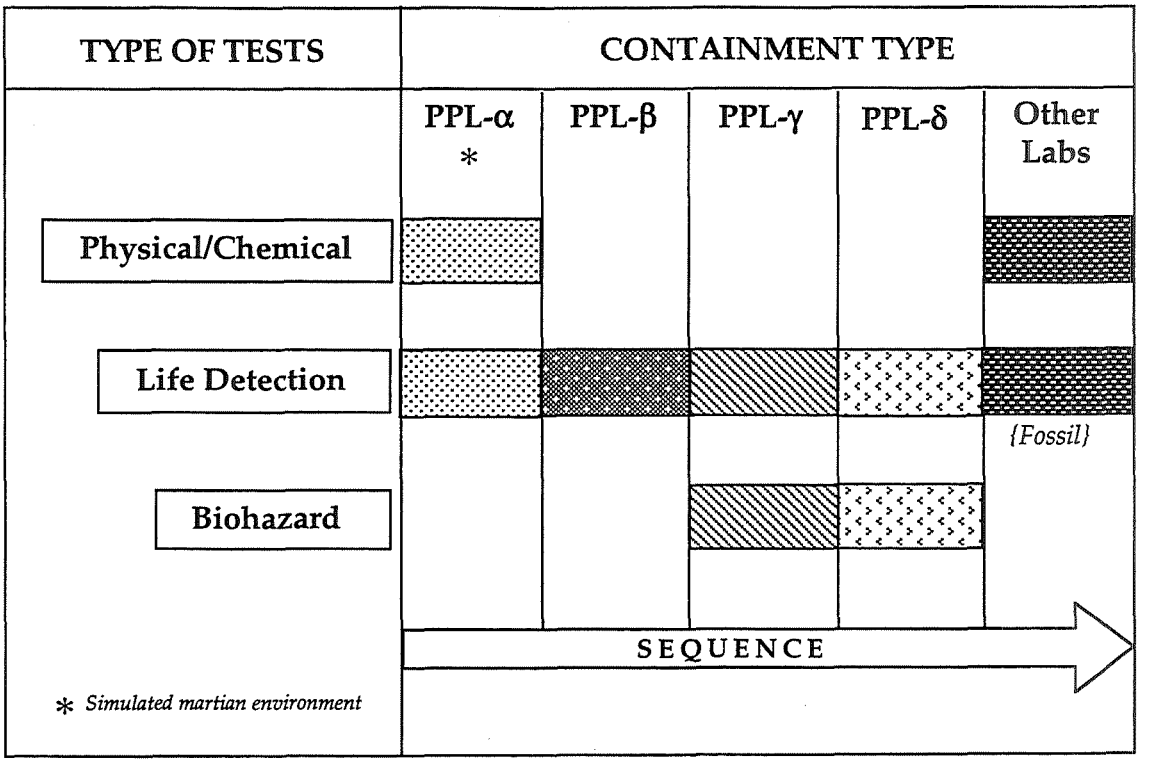

Figure WDP-5. Sequential containment requirements by test category
(see page 74 for the definitions of PPL-α, PPL-β, PPL-γ, and PPL-δ).

There are advantages to having a single facility that receives the samples and performs all functions up to PPL-γ before allowing transfer of some materials to PPL-δ facilities to complete the testing protocol. These advantages include a streamlined management and advisory structure, decreased sample volume for testing, fewer potentially exposed personnel to monitor, consolidation of appropriate experts at a single site, and diminished transportation and logistic concerns. Most importantly this approach assures that the samples are in the fewest number of facilities if they are found to contain life or a biohazard. The disadvantages of a single large facility are increased cost, possible decreased breadth of instrumentation, potential delays in recruitment or complications for visiting international partners, and lack of independent collaboration of test results.

In final analysis, the facilities required to implement this Working Draft Protocol, or its successors, represent the minimum set that should be provided for Mars Sample Handling. A variety of facility strategies can be pursued, depending on the availability of personnel and resources among the partners pursuing a Mars sample return mission. Further studies of this issue are required, as several of those strategies can provide for protocol completion as well as the optimal availability of the samples for scientific studies at the earliest possible time consistent with Earth safety.

Additional issues that must be addressed include:

- Completely define the PPL containment guidelines
- Continue to explore containment issues, options, and requirements (especially in collaboration with NIH, USAMRIID, and CDC), regarding refinements that will be necessary over the coming years to design or retrofit the appropriate and applicable biohazard containment facility.
- Develop schematics for a self-contained structure that could be placed in a BSL-4 laboratory and as a composite meet PPL-α containment requirements. This structure should be able to use remote robotics to handle the specimens.
- Develop a comprehensive list of equipment required for all proposed tests in the final protocol
- Anticipate the need to do some life detection under simulated martian environmental conditions while maintaining PPL-α/β containment.
- Put agreements in place with the any PPL-δ laboratories prior to receipt of Mars samples.

Environmental and Health Monitoring and Safety

Methods for monitoring the health and safety of the personnel of the SRF and the environment in and around the SRF, as well as at secondary sites if used, must be developed and implemented as part of the final protocol. This requires considering monitoring over time, beginning prior to the arrival of Mars samples, during work on the Mars samples at the SRF and at secondary sites, and assessing how long to continue monitoring.

Assumptions:

- The real risks associated with the Mars samples are unknown.
- The greatest potential risk is biological and includes "life as we don't know it."
- The potential exposure in the SRF will be of a small group of trained professionals until more information about the nature of the specimens is available.
- A high level of security for the SRF and the samples will be maintained as part of the PPL designation.

Recommended Principles for Development of Monitoring Program for SRF: Whenever possible, the monitoring plan should use existing regulations and standards. Since international teams will be working on the Mars samples, the regulatory standards from all participating countries should be reviewed and considered when developing the final monitoring plan. During the consideration of existing regulatory standards, the strictest standards, as appropriate for the anticipated hazards, should apply. Exemptions from existing regulations may be necessary, for example, differences in the protection of medical information between the participating countries. The leading principle for personnel monitoring and safety should be the optimal protection from the anticipated hazards for the individuals working with the Mars samples. Because of the unique nature of the potential hazards, additional controls than routinely used for hazard monitoring may be required. The monitoring plan should be designed to maintain a balance between the estimated risks to individuals, the environment or the general population and the personal impositions of the monitoring program. The monitoring plan should allow for cross-correlation of the data from the Life Detection and Biohazard testing of samples with the data from the monitoring of the SRF personnel and environment and allow for modification of either set of tests.

Potential Hazards: Five categories of potential hazards were considered: physical hazards, potential chemical hazards from non-biological toxins, biological hazards; failure or breach of containment; and psychological hazards. The physical hazards include hazards associated with equipment within the SRF labs and radiation from the Mars samples (which is expected to be negligible). The potential chemical hazards are predominantly from non-biological toxins. The biological hazards (including psychological) will clearly be the most difficult to monitor. The psychological hazards are those that may arise for personnel working under PPL conditions. Finally, monitoring of containment is a significant part of the monitoring program. Recommendations for monitoring for all hazards are as follows:

1. *Physical Hazard Monitoring (Radiation and Equipment):* Radiation is a standard hazard with well-established protocols for protection, handling and monitoring. To confirm the expectation that the Mars samples will not present a radioactivity hazard, a radioactivity measurement should be one of the initial measurements in the physical/chemical assessments. The measurement should be at a level appropriate to assess for a biohazard risk, and not to assess the absolute level of radioactivity present. Therefore, standard radiation safety protocols should be in place prior to the arrival of the Mars samples. If the radioactivity level does not represent a biohazard, then the monitoring for radioactivity can be discontinued, unless required for equipment used in the SRF. If a biohazardous level of radioactivity were detected in the Mars samples, then the radioactivity monitoring program would be continued. Other risks from equipment or facilities can be addressed by standard procedures of training and maintenance.

2. *Chemical Hazard Monitoring:* A chemical hazard from the Mars samples is most likely from non-biological, non-replicating toxins, if present. The presence of toxins will be assessed early in physical/chemical testing. If an unusual substance or chemical is identified, specific monitoring methods for that substance can be designed and the substance could then also be used as a marker for breach of containment monitoring.

3. *Monitoring of Containment:* Standard methods for monitoring of containment currently in use in BSL facilities can be adapted for use in PPL facilities and can be used to define a breach of containment or potential personnel exposure. If a breach occurs within the SRF, the breach can be corrected by standard procedures and personnel exposures can be assessed. If a breach occurs to the environment outside the SRF, a procedure should be developed to assess for possible environmental and/or human consequences. Procedures for handling a breach to outside of the SRF due to different causes (e.g., leak, disaster, security breach, etc.) should be considered in the development of the plans for handling a breach.

4. *Monitoring of the Environment:*
    - *Before Mars Sample Arrival:* A baseline assessment of the environment around the SRF should be made prior to the arrival of the Mars samples. The assessment should survey the pre-existing environmental conditions, and include an assessment of the water, air, flora, and fauna. This type of survey will likely be accomplished as part of the Environmental Impact Statement required prior to building SRF. During the baseline survey, sentinel species (microbes, insects, plants, animals) can be identified to use for monitoring for environmental changes. Consideration should be given to including some of the same organisms in

Biohazard testing. In case of noted changes in the environment around the SRF after arrival of the Mars samples, the Biohazard testing results could assist in determining if the changes were related to the Mars samples.

- *During Mars Sample Handling at the SRF:* Once the Mars samples are in the SRF, environmental monitoring could focus on the identified sentinel species and any novel components of the Mars samples, if identified. It may be useful also to track and record the weather conditions in area of SRF, for correlation in case of reports of a breach to the outside or any unusual events. If changes in the environment are noted on routine monitoring, assess if a breach has occurred. If a breach did occur, the breach procedures should be followed to reestablish containment and clean up any contamination. If changes in the environment are noted and a breach did not occur, assist with investigating the cause for the environmental change to establish that it either is or is not related to the SRF and Mars samples.

- *After completion of Life Detection/Biohazard Testing:* The level of continued environmental monitoring required should be reassessed based on the conclusions of the Mars sample testing protocols. Consideration should be given to maintaining the security and containment within SRF for assuring the proper curation of the Mars samples.

5. *Monitoring of the SRF Personnel:*

- *Before Mars Sample Arrival:* A process of certification for people who will work in the SRF should be developed that includes education about procedures and risks for employment, security clearance, and medical examinations and tests. Clear inclusion and exclusion criteria based on the results of the certification procedures should be developed prior to the hiring of personnel.

  Baseline medical evaluations of personnel should use the existing medical evaluation standards appropriate at the time the evaluations are performed. Since the SRF will be functional for a period of time prior to the arrival of the Mars samples, monitoring before the arrival of the Mars samples could include several evaluations (a period of two years was proposed). Recommended baseline evaluations include a medical history, physical examination, tests on the person (e.g., chest X-ray), and tests on samples from the person (e.g., blood and urine). All testing should be as non-invasive as possible and maintain a balance between estimated risks from the Mars samples and the risks associated with the tests. Specimens should also be archived for future comparison, if needed, and may include serum, lymphocytes, semen and/or hair. In addition neuro-psychological evaluations using standard testing techniques with well-established interpretation methods should be administered. Symptom data should be obtained using standardized instruments such as the Millennium Cohort survey (USA) or the GAZEL Cohort survey (France).[54]

54. The exact survey instrument has not been identified, but it would be possible to use currently existing surveys similar to the Millennium Cohort Study (USA) or the GAZEL Cohort survey (France), sponsored by the US Dept. of Defense and INSERM, respectively. Current information about these two surveys, respectively, may be found online at: <http://www.millenniumcohort.org> and <http://www.gazel.inserm.fr>.

- *During Mars Sample Handling at the SRF:* A schedule for regular evaluations of personnel should be established, using the same evaluation methods as used for the baseline data collection. Procedures for standard medical management of personnel illnesses should be available either on site or with adequate transportation to a medical facility, as needed. Intervention should be correlated with an identified, or risk of, exposure to the Mars samples. If an exposure occurs and the exposed individual has or develops symptoms, the person should be transferred to a medical facility with BSL-4 containment capabilities, until proper assessment of the individual is accomplished. If an exposure occurs and the individual does not have or develop symptoms, procedures for quarantine of the individual should be developed with specific guidelines as to the length of quarantine required if the person remains asymptomatic. If an individual becomes symptomatic and there is no evidence of an exposure, the individual should be treated as appropriate for the symptoms and monitoring should continue as prescribed by the Working Draft Protocol.

- *After Completion of Life Detection/Biohazard Testing:* The question of how long to continue monitoring has to be addressed. Certainly the duration of monitoring will be influenced heavily by the outcomes of the Life Detection and Biohazard testing. Several factors may need to be considered in this decision, such as the protection of the workers versus the protection of the general population. Clearly articulated decisions will be needed on whether to have lifetime surveillance for the personnel or a mandatory period followed by optional reporting, if the risk was determined to be low. Certainly monitoring may become optional if the samples are deemed safe by the Life Detection and Biohazard testing. Whether or not surveillance is needed for relatives or people living close to the workers should be considered. A distinction should be made between monitoring for risk management and continued collection of data for a research study. The interpretation of personnel evaluations may require the use of a control group or population-based estimations of frequencies of different events. If so, sources for this information should be defined.

Monitoring at Secondary Sites: The level of monitoring to be used at secondary sites receiving and working on portions of the Mars samples should be based on the results of the Life Detection and Biohazard testing. If the Mars samples are still potentially hazardous or their biohazard status is unknown, several points should be considered in the development of a protocol for monitoring at secondary sites. First, secondary sites should be identified prior to the arrival of the Mars samples, to allow for pre-certification of personnel and their baseline data gathering. Second, all distributions should be tracked and procedures for monitoring of containment at the secondary sites should be developed. Third, consider monitoring personnel at secondary sites using the same protocols as used at the SRF. The number of personnel at secondary sites is expected to be a small number of individuals.

If the Mars samples are deemed safe either through "sterilization" or by biohazard test results, then methods should be used for tracking all sample distributions and all individuals in contact with the samples. In this case, only event reporting is needed.

Database Issues: A central database facility with data analysis capabilities and procedures should be used for environmental data (e.g., baseline, monitoring), personnel data (e.g., baseline, in-process, follow-up), secondary site data and sample tracking data. Procedures for regular data analysis and

reporting should be developed. Access to and confidentiality of the data should be defined and assured. Data analysis should distinguish between surveillance and research, with consideration given to the need for ethical review and approval for research protocols.

The Following Points Need Further Consideration:

- Criteria for inclusion/exclusion of personnel to work at the SRF or at secondary sites.
- The time frame of personnel monitoring: lifetime versus limited period (according to hazards).
- If long term monitoring is implemented, what parameters need to be monitor on a long term basis.
- Need for informed consent for the testing and possible long term monitoring.
- Should monitoring be restricted to relevant public health measures as opposed to extending the Working Draft Protocol to allow for epidemiological research.
- Level of medical facilities needed at the SRF.
- Level of baseline testing and monitoring for secondary site workers as compared to workers at the SRF.
- Protection of individuals from life or health insurance discrimination.
- Procedures for database management and data analysis with consideration of confidentiality and security issues.

Summary: Monitoring methods for personnel and the environment should be developed considering international regulatory, cultural, and ethical issues. The radiation and chemical risks are considered of low probability and can be assessed early in the chemical testing procedures to reduce the monitoring burden. Procedures must be developed for database management and data analysis with assurances for confidentiality and security of the data. Procedures for monitoring personnel should include procedures for education and certification.

Personnel Management Considerations in Protocol Implementation

Staffing the Sample Receiving Facility(-ies) can be accomplished in a number of alternative ways. For example, scientists can be recruited to fill permanent positions at the SRF or could be selected through a competitive grants program for work at the SRF. Considering the variety of personnel categories that will be required to accomplish varied tasks during design, building and operation of the facilities, as well as during implementation of the Final Protocol, it will be advisable to utilize a variety of different selection and hiring processes. Personnel should be hired progressively during the development of the project and the facility(-ies). At least initially, the functions and responsibilities of the director's position may be carried out by appropriate committees until about five years before the return of samples from Mars. In the event that more than one facility is used, the required methods and procedures outlined in the Protocol should be applied beyond the SRF to any facility or site handling martian samples during the implementation of the Protocol. Because researchers and the public worldwide will have interest in returned martian materials, the international character of the program should be respected throughout the whole process.

Figure WDP-6 presents a high level schedule and overview of the process from now until the samples return to Earth. The functions, staffing requirements, and organization that will be needed to design, build, and operate a Mars Sample Receiving Facility are further elaborated in Figures WDP-7, -8, and -9; these figures describe proposed staffing and organization at 10, 5, and 3 years before the arrival of actual samples at the SRF. These proposed management, staffing, and organizational frameworks amounts to working hypotheses that have been based on the following assumptions:

- The protocol must be fully and successfully tested before the actual handling of martian samples. The exact makeup and sequence of Experiment Verification Tests (EVT) are TBD.
- It's estimated that a complete series of EVTs will last approximately 6 months and one complete series must be successfully demonstrated before actual handling of the returned samples. The first EVT series must begin no later than 18 months before the returned samples arrive at the SRF in order to allow enough time to adjust and repeat the series if necessary (at least 9-10 months before experiments begin on actual returned samples).
- These EVTs are consistent with the recommendation of the SSB (1997) and earlier Workshops in this Series that the SRF be operational two years before the arrival of the actual Mars samples. These EVT are part of the normal operational testing.
- Based on experiences at other BSL-4 laboratories in the United States and France, no less than one-year is required to properly staff and train the technical and scientific personnel.
- Commissioning of the SRF, which must be performed in parallel with the staffing and training, will last a least 18 months.

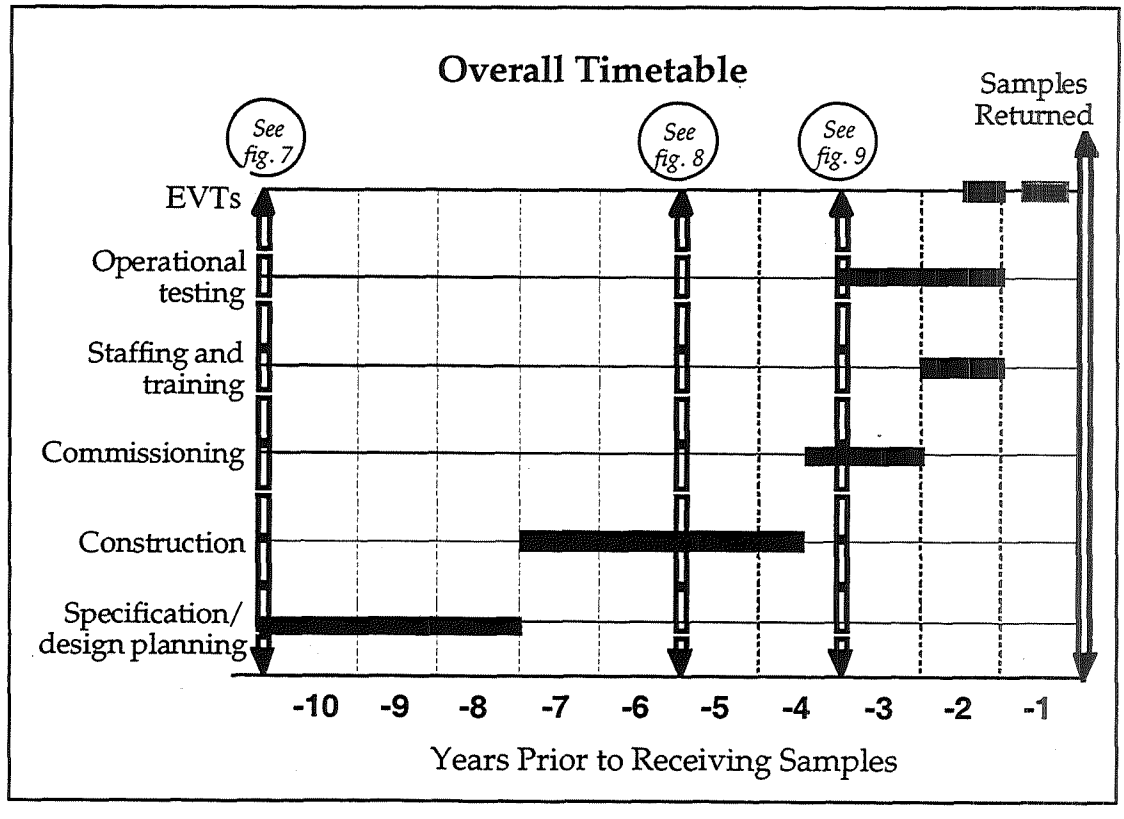

Figure WDP-6. Example overall timetable of activities required to design, build, and operate the SRF. Double-headed arrows indicate times described in subsequent figures. (EVTs = Experiment Verification Tests).

- In order to accommodate the staffing, training and commissioning requirements of the SRF, construction of the facility must be finished 3 years before the actual operations. From past experiences in both France and the US, construction of the facility itself will require 3 years.

- It is estimated that about 3 years will be needed to develop design specifications and plans for the SRF and obtain necessary authorizations for the facility. To accommodate all the activities necessary to design, build, and operate an SRF, the entire process must begin fully ten years in advance of sample return.

To illustrate one approach to staffing and organization to meet facility and protocol requirements, specific details related to the recommended staffing and organizational plans are provided below. Accordingly, these are not intended to be fixed requirements in this Working Draft Protocol, but are intended to provide a surrogate structure on which to base future staffing plans.

10 Years in Advance

As soon as the decision is made to build and/or update a Mars sample receiving facility, approximately 10 years before the actual operations, four positions should be staffed in order to allow the preparation of specifications for future activities and to allow a substantive review of the design of the facility. Figure WDP-7 shows the key positions 10 years prior to sample return: the Project Manager/Director, a Director for Administration, a Project Scientist/Director for Science, and an Environment, Health and Safety Officer. The Director, who is responsible for the overall sample handling project implementation, will have the assistance of an Oversight Committee that will monitor

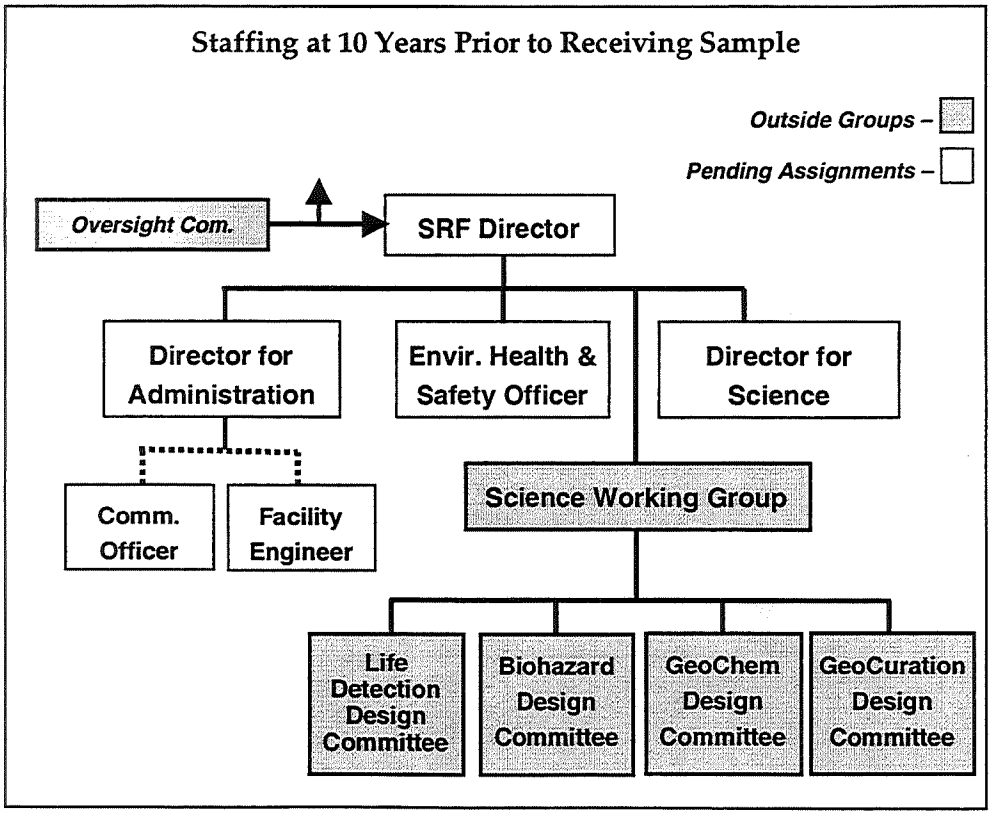

Figure WDP-7. Top-level staffing requirements and structure of the SRF at 10 years prior to arrival of the returned sample(s) (permanent positions are in plain boxes; committees are in colored boxes).

progress and assure the compliance of the project with the Protocol and with whatever science requirements are also to be implemented in the Facility. In this example, it is anticipated that the initial Director will have a scientific facility engineering background, and that a transition to a Director with a science background would be made after Facility construction is assured. The Director will be assisted by the Environment, Health and Safety Officer to ensure that the actual design requirements related to these critical topics are properly implemented. A Director for Administration will focus on budget and staffing issues, and the development of the staffing plan to cover the life of the project.

Additional engineering support (e.g., the Facility Engineer) would be added as necessary. The Project Scientist/Director for Science will coordinate the work of scientific committees and working groups that will develop science specifications and support the design process for their respective disciplines or areas. Also at this point in the project, a Communications Officer should be available, at least on a part-time basis, to ensure attention to risk communications and outreach – keeping the community informed and identifying and answering questions regarding the SRF.

5 Years in Advance

At roughly midway through the construction of the facility, the Scientific Discipline Heads should be hired for each required scientific discipline (see Figure WDP-8). These managers will ensure that construction is properly completed to accommodate the specific needs of their disciplines.

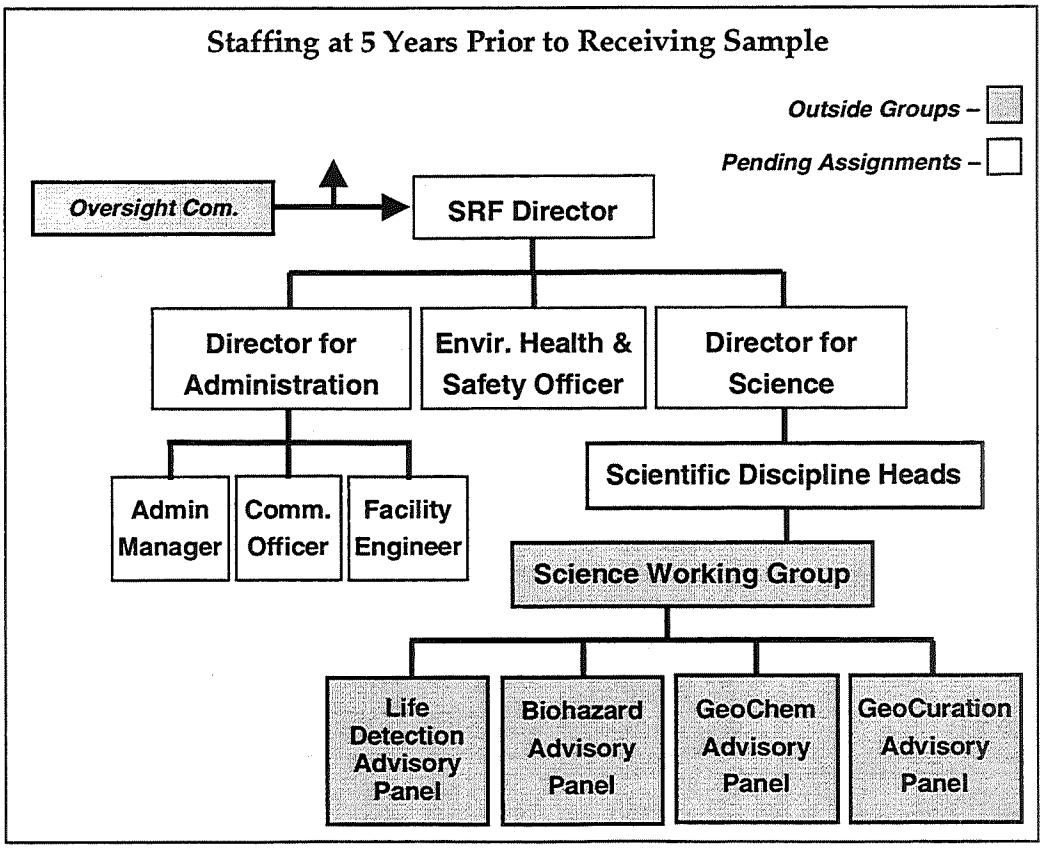

Figure WDP-8. Top-level staffing requirements and structure of the SRF at 5 years prior to arrival of the returned sample(s) (permanent positions are in plain boxes; committees are in grey boxes).

With the help of experts working as part of the scientific working group and discipline advisory panels, they will complete the general and specific operating procedures to handle the martian samples and the training program for staff to be hired. At this point, a Facility Administrative/Staff Manager will also be hired to assist in the hiring of the technical staff and prepare for future administrative and personnel needs of the facility.

3 Years in Advance

In order to have a fully operational facility two years before samples are returned, the final staffing and training of various operational positions must begin three years prior to actual operations (see Figure WDP-9). At this time, required supporting groups such as an Institutional Bio-Safety Committee (IBSC) and an Institutional Animal Care and Use Committee (IACUC) will be formed, as well as necessary support staff to support facility operations, administrative functions, communications, and safety program implementation.

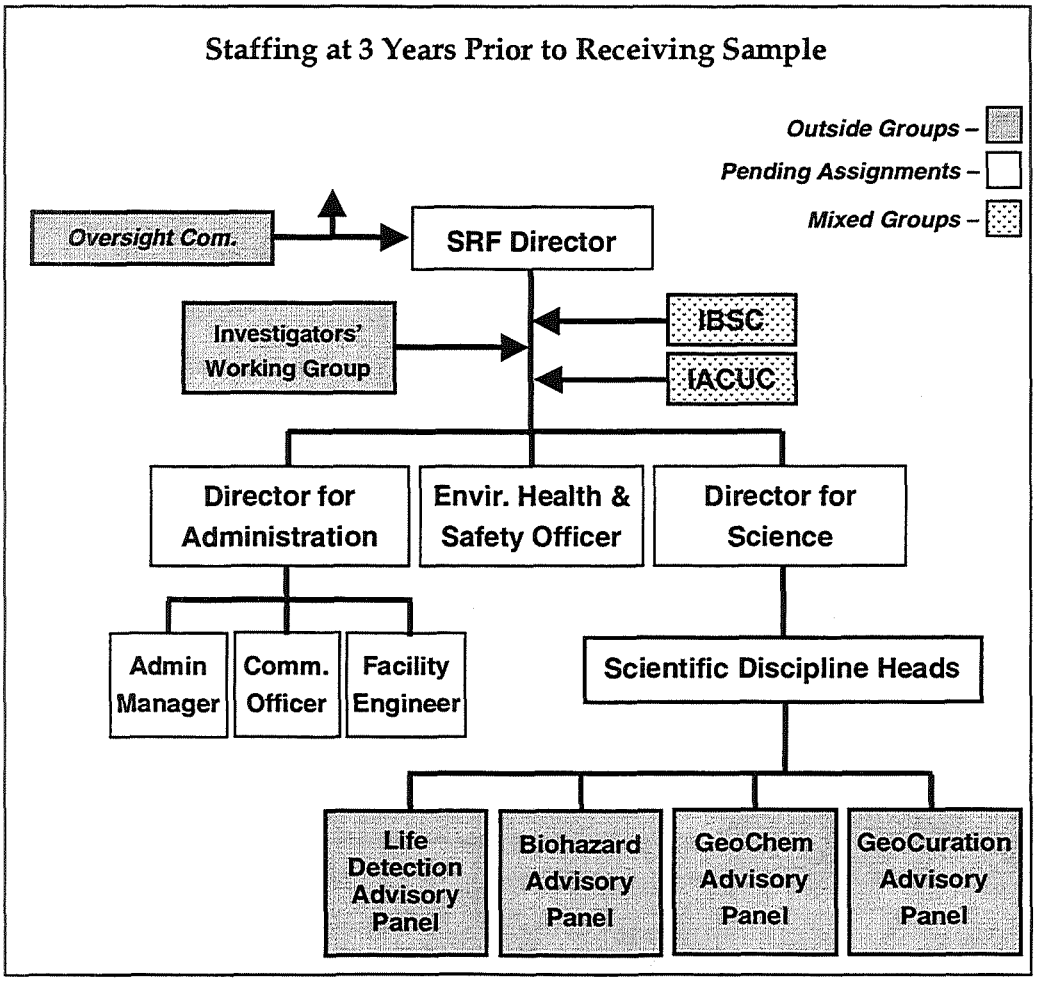

Figure WDP-9. Staffing requirements and structure of the SRF at 3 years prior to arrival of the returned sample(s) (permanent positions are in plain boxes; committees are in grey boxes).

Also at this time, it is anticipated that the ad hoc Science Working Group (which would deal with both science issues and issues of planetary protection protocol compliance) would be supplanted by an Investigators Working Group that would be selected through an open solicitation that would provide for scientific investigations to be accomplished within the facility. The relationship of these selected science investigations to the accomplishment of Protocol Objectives may be close or distant, depending on the strategy to be undertaken by the Project to implement the protocol. From the beginning of the process, three different kinds of committees should be installed to help the Directors and Scientific Discipline Heads in overseeing their changing responsibilities.

Scientific design panels will be specialized in the four disciplines (Life Detection, Biohazard, Geo-curation, and Geochemistry). The members, who will be prominent scientists, will be designated by the agencies. These committees will prepare the design, review the project and oversee the project to ensure the facility can operate consistent with the operational aspects of the planned Protocol. As soon as the Scientific Discipline Heads are hired, these committees will shift to become Discipline advisory panels helping them.

The Science Working Group will be charged with helping to guide the overall project during the construction phase, to provide recommendations and expertise in assuring its compliance with sample scientific requirements and the Protocol. The members of the SWG are chosen from an ad hoc set of "Mars Scientists" representing the required disciplines and expertise. Later, they are replaced by the Investigators Working Group, who will be the selected Principal Investigators from an open competition seeking proposals for sample analysis activities within the Facility.

Finally an oversight committee of 12 to 15 members will be selected by the Program leadership, perhaps from NASA's PPAC and the French PPC. These committees will be in charge of reviewing the overall process and the proposed measures to comply with the requirements of the Final Protocol. The committee will report to the Program Management and the Planetary Protection Officer, above the level of the Project Manager/Facility Director – though it is expected that they will interact directly with that Manager on a regular basis.

Membership on the various committees will be staggered to insure an appropriate turnover without loosing the memory of the project. Agencies involved with the SRF should set up an international search committee for recruitment of the Directors, various functional managers, the Facility Engineer and the Scientific Discipline Heads.

Three major issues will require further consideration in the overall staffing of the SRF:

1. Currently, no one has experience in simultaneous operations or activities in combined BSL-4 and clean room conditions as will be needed for PPL-α through PPL-δ. The advice of experts from the high-potency pharmaceutical or the micro-process industries would be helpful.
2. Details on the optimal staffing mix at the SRF must be considered further. It is not clear what mix of government employees, semi-permanent staff employees, outside contractors, and guest scientists will be needed to staff the facility and implement the Final Protocol. In planning for facility staffing and operations, international access and participation should be considered throughout the process.

- In order to comply with planetary protection constraints and protocol requirements, a sustained and adequate budget will be needed throughout the design, building and implementation phases of this project.

Contingency Planning for Different Protocol Outcomes

Developing contingency plans for different protocol outcomes will require anticipating how the scientific community might interpret test results and react under a variety of possible scenarios following the return of martian samples. In addition to considering how to interpret possible scientific results, it will be important to plan how to respond in the face of possible breaches in containment. Recommended response to various likely scenarios are discussed below:

Organic Carbon: It is likely that carbon will be found in sample materials. The sensitivity of current and future methods will be very high, so that at least some level of contaminants should be detected, and perhaps carbon compounds from Mars, as well. The existing base of knowledge on meteorites and other material collected from space will be useful in providing baseline information to help guide these investigations. Since the Viking results focused on volatile organics, further attention to the question is appropriate. *In situ* measurements of non-volatile organics on missions prior to the sample return mission would be useful to gauge predictions of anticipated sample organic content.

Extant Life or Biomarkers Positive: If extant life or evidence of biomarkers are detected in the samples, all work on the samples will continue to be done in strict containment facilities until more definitive data can be gathered [see release criteria, above]. Maximum effort should be made to determine if the positive results are originating from Earth life or Mars life. Information management will become an issue both for scientific communication and in shaping the debate among scientists. It will be important to plan for how initial information, with its attendant uncertainties, should be disseminated to the public.

Non-Earth Life Confirmed: In keeping with the SSB recommendations *[SSB 1997]*, and the stated release criteria, sample materials will be released from containment only if they are shown to contain no extraterrestrial life-forms, or they are sterilized prior to release. If non-terrestrial life is confirmed, a previously constituted scientific oversight committee will need to review the steps taken in support of the Draft Protocol, the Draft Protocol itself, and ongoing provisions for containment. If a portion of a sample is confirmed as positive for non-terrestrial life, subsequent testing and analyses on all sample materials will continue in containment. This means that all physical, chemical, and geological characterization, as well as Life Detection and Biohazard tests requiring non-sterilized material should continue to be done in strict containment, either in the SRF or in any other test facilities that may be used. Experimentation on methods to sterilize samples containing the newly discovered life should begin in conjunction with investigations of appropriate culture conditions. Once appropriate sterilization techniques can be validated, detailed plans for distribution of samples can be developed or revised in order to meet the established or revised scientific objectives. Management issues will include administrative and technical procedures for scientific study and curation, as well as informing the public.

Although it is premature to develop specific recommendations at this time, it is possible to identify issues that will need further discussion in advance of sample return. The concerns fall into three broad categories: Science and Testing; Facility and Technological; and Policy and Administrative:

Science and Testing: Confirmation of a preliminary discovery of martian life should require a careful reconsideration of results from many parts of the Draft Protocol, ranging from a review of preparation,

scanning and testing methods, to verification of biocontainment materials and sterilization techniques, to a reassessment conditions for banking, storage, transportation and curation. In addition, it will be important to understand the culture and environmental conditions required to maintain and perhaps to grow the new life-form to obtain more material for study in the lab – and what precautions are needed in the process. In addition, it will be important to review the final protocol to recommend modifications in physical, geological, and chemical tests of samples, adding or deleting tests as needed

Facility and Technological Concerns: Questions about the adequacy of the SRF to maintain the new life form must also be addressed, including the possible need to add equipment, change operations, review emergency plans or upgrade the facilities because of what has been found. Concerns about security should also be reconsidered, especially in view of the potential disruptive activities of any 'radical' group that may be opposed to sample return. The advisability of allowing distribution of untested sample material outside the SRF may need to be reconsidered, as well.

Policy and Administrative Concerns: If martian life is detected, both short-and long-term policy issues will arise. The short-term listing of concerns relates to procedures regarding access to and distribution of sample materials, as well as to the publication and review of research findings. In anticipation of the discovery of extraterrestrial life, it will be advisable to develop an organized communication plan. This should be done well in advance of the event, in order to avoid a frenzied, reactive mode of communications with government officials, the scientific community, the mass media, and the public. Any plan that is developed should avoid a NASA-centric focus by including linkages with other government agencies, international partners, and external organizations, as appropriate. It will also be advisable to anticipate the kinds of questions the public might ask, and to disclose information early and often to address their concerns, whether scientific or non-scientific.

In the long term, the discovery of extraterrestrial life, whether *in situ* or within returned sample materials, will also have implications beyond science and the SRF *per se*. Such a discovery would likely trigger a review of sample return missions, and plans for both robotic and human missions. Legal questions could arise about ownership of the data, or of the entity itself, potentially compounded by differences in laws between the United States and in the home countries of any international partners. In any event, ethical, legal and social issues should be considered seriously. Expertise in these areas should be reflected in the membership on appropriate oversight committee(s).

Contradictory/Inconsistent Results: Given the number of techniques, spanning several scientific disciplines, it is very likely that contradictory or inconsistent results will be found. Differences in the sensitivity of methods will exist and confidence in the reliability and level of experimental controls will differ among procedures. It is important to stress the need for replication of experiments and duplication of results among multiple sites to add confidence to the results assessed. In addition, it will be important to follow a strict scientific procedure for interpreting data and making decisions about sample materials. There is a need to involve multidisciplinary experts and groups in the overall decision making process as well in devising procedures for drawing conclusions, certifying results, and deciding whether samples are safe enough to be released to lower containment levels.

Application of Release Criteria: According to the COMPLEX report *[SSB 2002]:*

> *"If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, release of un-sterilized aliquots of the samples for study beyond the confines of the Quarantine Facility is justified."*

The stated goal of this Protocol Workshop Series was to design a protocol to test the sample(s) for biohazards and the presence of martian life that could be applied to ensure that a sample is safe to be released without sterilization. The release criteria listed above are consistent with this recommendation, but with the additional requirement to complete biohazard testing in addition to the tests for organic carbon (and other, similar life-detection testing).

Arguments have been advanced that suggest that a sterilization step be added to the protocol for the release of any materials, for "good measure," even if the samples are devoid of organic compounds and do not demonstrate any biohazard. Based on the arguments advanced, pro and con, this additional step is not contained in this Working Draft Protocol. Central to an understanding of the arguments is the question of risk – Can *any* protocol be guaranteed to be absolutely risk-free? If not, what is an acceptable level of risk (for example, one that approximates the risk from the natural influx of martian materials into the Earth's biosphere)? And is there any treatment method that can eliminate all risks from the returned samples while preserving them for the detailed scientific study envisioned by scientific community? Clearly, the issue of sterilization will require serious additional attention and research well in advance of sample return. Likewise, the safety of releasing materials that have passed both life-detection and biohazard testing should be carefully challenged through a rigorous quality assurance program applied to the completion of the final protocol.

Breach of Containment: Anticipating a containment breach and planning for such an event is an essential element of facility management. The responses to a breach will depend on where it occurs and what happens. Conceivably, it could occur in an area with a high population density or in a remote location. The breach could be a result of an accident or a crime – as a result of activity either outside or within containment. The consensus of the Sub-group was that we know basically how to handle breaches based on long term experience and emergency plans for handling pathogenic biological material under BSL-3 and BSL-4 containment. Additional information for responding to breaches and containment problems has been gained through decades of experience in handling lunar and extraterrestrial materials.

Clearly, an emergency plan will be needed well in advance to develop recommended responses to various breach scenarios. The first steps would involve investigation of the degree of compromise, considering both biosafety and sample integrity. Full documentation of any breach event will be required as well as identifying the degree of sample compromise, what organizations or personnel should be involved in all phases of a response, and how notifications and communications should be handled. The plan should focus on all aspects of mitigation, cleanup, and recovery from perspectives of both biosafety and sample integrity (e.g., decontamination of the area; sample recovery, re-packaging and labeling as compromised, or destruction if required, etc.)

Maintaining and Updating the Protocol

The COMPLEX report *[SSB 2002]* also recommended:

> *"A continuing committee of senior biological and geochemical scientists that includes appropriate international representation should be formed, and charged with reviewing every step of the planning, construction, and employment of the Mars Quarantine (and initial sample handling) Facility. The committee should be formed during the earliest stages of planning of a Mars sample return mission. Members of the committee should also participate in the design of the spacecraft and those portions of the mission profile where biological contamination is a threat."*

The protocol implementation and update process will require establishment of a number of expert oversight and review committees; re-evaluations of proposed plans at key points in time before sample return; and open communication with scientists, international partners and the public about risks, benefits and plans. The scope of the task is summarized in Figure WDP-10. A narrative explanation of recommendations and activities in the process is provided in the text that follows.

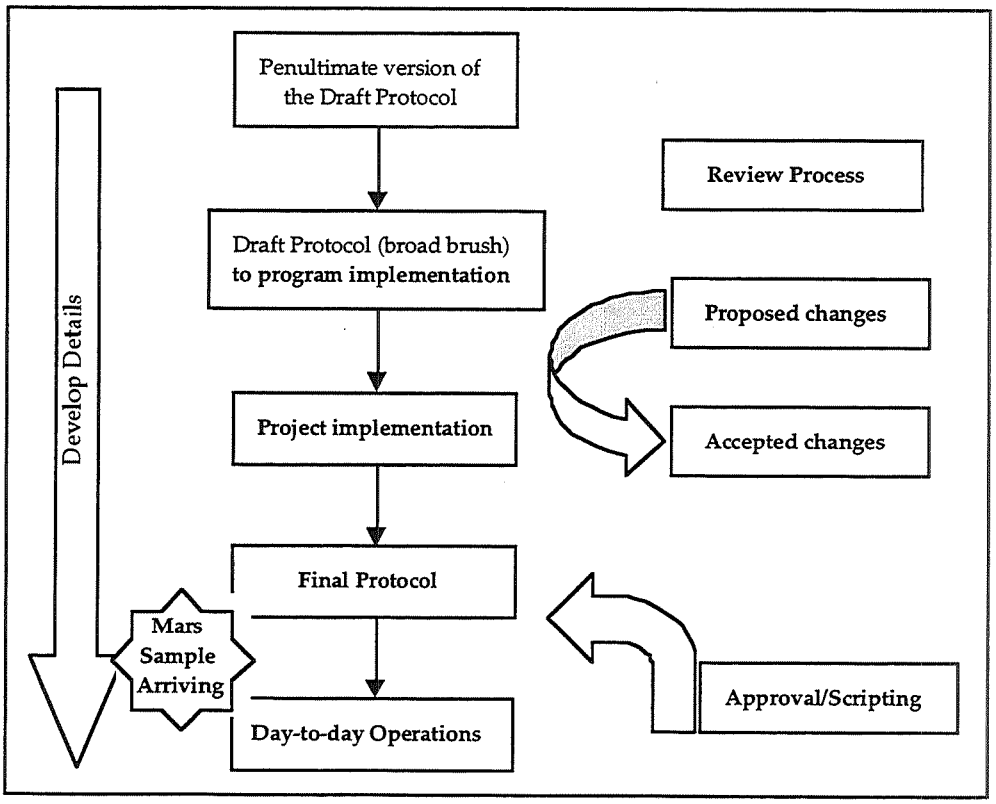

Figure WDP-10. Protocol Implementation and Update Process.

Final Scientific and Policy Reviews: The final review of the protocol document should be subjected to the highest degree of scientific scrutiny and evaluation. The evaluation should be conducted jointly by scientific organizations from both the United States and France to avoid prolonged negotiations and resolutions that may arise when such reviews are conducted separately. This review should probably occur at the level of the National Research Council in the United States, and its equivalent scientific

organization in France. The French members of this Sub-group agreed to investigate which of the French institutions is most appropriate (among the French institutions discussed were CNRS or representatives of various Etablissements Publics à Caractère Scientifique et Technique (EPST), including but not exclusively CNRS or Académie des Sciences. Final decisions about which institutions should be involved in scientific reviews is TBD.

Clarity of Meaning and Terminology: Clarity of meaning is essential to the implementation of any process especially when the process involves international agreements. Therefore, absolute consistency should be used in the language for any documents and charters associated with the final protocol. When the actual definition of a word or phrase is in dispute, reference should be made to those definitions or meanings that are standard and accepted when interpreted at the international level. Clarity in terminology will be especially important when describing levels of containment so as to avoid confusion caused by mixing United States and French definitions of BSL, PPL, and P4 containment.

Ethical and Public Reviews: Evaluations of the proposal should be conducted both internal and external to NASA and CNES and the space research communities in the nations participating in the mission. An ethical review should be conducted at least at the level of the Agencies participating and these reviews made public early in the process (in France, the National Consultative Bioethics Committee, CCNE, is the appropriate independent organization). The final protocol should be announced broadly to the scientific community with a request for comments and input from scientific societies and other interested organizations. Broad acceptance at both public and scientific levels is essential to the overall success of this research effort.

Future Modifications to the Protocol: When a final protocol has been adopted and approved by a consensus of appropriate scientific organizations, few changes should be made to its content. Changes should be made as scientific information, methodology and/or technology improve between the time of the approval and the actual physical implementation of the final protocol within the SRF laboratories. Changes in the final protocol methodology or technology may be considered if a proposed change would meet the following criteria:

- increases the sensitivity or selectivity of the test,
- reduces the length of time necessary for a test without a reduction in sensitivity or selectivity,
- reduces the complexity of the sample handling process,
- increases the overall safety of the process,
- reduces the chances of contamination to the sample or the environment,
- reduces the cost of the process, or
- represents a new technology or method that has the broad general acceptance of the scientific community.

Advisory Committees and Expert Panels: Changes to scientific methodology and instrumentation are inevitable due to the long development time envisioned for this mission. This necessitates long term, consistent input and advice from the external scientific communities of all the partners engaged in the process. To facilitate this process, it is recommended that a standing Planetary Protection Advisory

Committee (PPAC) be appointed in the United States to provide input to the Planetary Protection Officer and that a similar standing committee (Planetary Protection Committee, PPC) be appointed in France as tentatively planned.

Standing joint (French and U.S.) working committees or specialized expert panels should be appointed with appropriate expertise to provide support and advice to the United States PPAC and French PPC in each of three specific areas: technical processes, scientific procedures, and safety/biosafety issues. To provide the highest level of support to the process, these groups should be individual panels comprised of members with expertise in a particular area of concern. Individual experts should be limited to a single panel. The overall membership of the committees and expert panels should meet the specific needs of the Agencies and should represent the scientific goals of the Agencies and the external science communities. Their work should aim at providing the respective agencies with information essential to the success and safety of the Mars sample return missions. These panels and committees may function jointly or independently depending on the specific need.

The PPAC and French PPC will receive the annual reports of the three panels, which will also provide annual written reviews to the NASA Planetary Protection Officer and, in France, to the appropriate Minister to whom the committee reports. These reviews should include relevant operational issues and concerns and provide risk assessments of the technical processes, scientific procedures, and safety/biosafety plans and processes. These reviews should be made available to scientific and professional organizations with interests in the mission activities.

Communications: Unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels. Therefore a communication plan must be developed as early as possible to minimize the dissemination of misinformation and to provide the highest level of public assurance about the issues addressed by the mission. Communications should clearly inform about the extensive efforts to protect the environment, health and safety through facility designs, procedures, and personnel training. This information on risk management and planetary protection should be balanced with education/outreach about the anticipated benefits of Mars exploration and sample return from the scientific perspective. The communication plan needs to address the concerns of both the scientific community and those external stakeholders who will raise valid concerns on behalf of the world's population. In order to minimize long-term criticism and concerns, it will be important to inform the public openly and honestly about all aspects of the mission in a way that provides accurate, timely details about scientific benefits, expectations, risks, and uncertainties. In particular, both the public and scientific community should be informed of results during Life Detection and Biohazard Testing at appropriate times in the process based on procedures and criteria (e.g., level of certainty, consensus or majority, etc.), for determining how observations and data will be designated as results suitable for formal announcement. Details about who will be in charge of this communication plan and the release of information are TBD.

Flow Charts and Timelines: In order to assure the rational utilization of both the facilities and sample materials, development of appropriate flow charts and time lines will be needed to coordinate the complex series of interrelated procedures. Safety issues must be prominent at all significant decision points in the process (e.g., release from containment, downgrading to lower level of containment) This means that everybody has knowledge of the critical points for these decisions and understands

they are not negotiated on the fly. Such flow diagrams are intended to coordinate complex testing and inclusion of all required elements especially those concerning biosafety and biohazards leading to the sharing of sample material with the external scientific communities. Such flow charts, in addition to time lines, procedures and process, should also include key decision points for changing the status of the sample to a less restrictive PPL and to proceeding in a particular direction along branches of the decision tree. Each such chart should contain an incorporated risk tree and assessment process.

Workshops/Reviews: The need to change schedules and procedures may be anticipated during the time between now and sample return. To provide assurance that rules exist between the involved international partners and the scientific communities, two workshop/reviews should be scheduled prior to sample return to Earth in order to reaffirm details about process, methodology, safety and release criteria. The first review should be conducted at the conclusion of the facilities design phase to determine if the physical structure meets the scientific and safety standards as defined within the specifications. In addition, the first workshop should review the existing procedures that will be conducted within the facility to confirm the specific flow chart outlining the approved sequence of tests and analyses. A second similar workshop/review should occur after the samples have been collected on Mars but in advance of their actual return to Earth for evaluation. Details about who should coordinate these workshop/reviews and modify schedules or procedures are TBD.

Preparations and Processes for Decision Making about Release of Samples: It will be important to make advanced preparations for data interpretation and decision making in an organized way. These preparations will be especially critical in the event that a distinctly martian life-form is found within the returned samples. While it is impossible to develop details of the final protocol at this time, it will be crucial to have considered how decisions will be made, by whom, and based on what principles if an extraterrestrial life-form is discovered. A specific committee should be established at least a year ahead of sample return to develop contingency protocols and processes that will be in place if and when a martian life is found and verified.

It is likely that protocol test results will not lead to unanimous decisions in all instances. It will thus be important to have a review and approval infrastructure for handling decisions about whether or not to release sample materials from containment, or reduce containment to a lower level, upon completion of protocol tests. Addressing the overall decision making process in a formal manner will be critical for drawing conclusions, certifying results, and deciding whether samples are releasable or not. Any decision to release samples should involve selected multidisciplinary experts and groups, as well as an Interagency Committee on Back Contamination (ICBC) similar to the one used during the Apollo program. The U.S. PPAC and French PPC should be involved in reporting to relevant bodies in their respective countries. Details on the structure(s) associated with decision making are TBD.

The organizational structures, management plans, charters and reporting lines for many of the proposed committees and groups will need to be developed in the coming years. Many questions cannot be resolved until additional details on facility design, operational logistics, mission architecture or anticipated schedules are made available.

APPENDIX B
WORKSHOP SERIES ASSUMPTIONS

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

To keep the Workshops focused, a set of basic assumptions were provided to guide and constrain deliberations; these assumptions were:

1. Regardless of which mission architecture is eventually selected, samples will be returned from martian sites which were selected based on findings and data from the Mars Surveyor program missions.
2. Samples will be returned sometime in the next decade.
3. Samples will not be sterilized prior to return to Earth.
4. When the Sample Return Canister (SRC) is returned to Earth, it will be opened only in a Sample Receiving Facility (SRF) where samples will undergo rigorous testing under containment prior to any controlled distribution ('release') for scientific study.
5. The amount of sample to be returned in a SRC is anticipated to be 500-1000 grams.
6. The sample will likely be a mixture of types including rock cores, pebbles, soil, and atmospheric gases.
7. The amount of sample used to determine if biohazards are present must be the minimum amount necessary.
8. Samples must be handled and processed in such a way as to prevent terrestrial (chemical or biological) contamination.
9. Strict containment of un-sterilized samples will be maintained until testing for biohazards and Life Detection is accomplished. Sub-samples of selected materials may be allowed outside containment only if they are sterilized first.
10. The SRF will have the capability to accomplish effective sterilization of sub-samples as needed.
11. The SRF will be operational two years before samples are returned to Earth.
12. The primary objective of the SRF and protocols is to determine whether or not the returned samples constitute a threat to the Earth's biosphere and populations (not science study *per se*) and to contain them until this determination is made.

APPENDIX C
WORKSHOP 4 AGENDA

Day 1: Tuesday 5 June 2001

8:30 am	Introductions, opening remarks
9:00	Lecture 1 – Summary of Workshops 1, 2, 2a, and 3 (*M. Race*)
9:30	Lecture 2 – Results: U.S. NRC Mars 2001 Sample Handling Study (*J. Rummel*)
10:15	Break
10:30	Lecture 3 – Presentation of Strawman Comprehensive Protocol (*Team*)
11:30	Objectives of Workshop 4, Sub-group charters, etc. (*J. Rummel*)
12:00 pm	Lunch
1:00	Sub-group deliberations • Sub-group 1 – Review, assess, and adjust protocol for sample container processing, sample preparation, and physical/chemical analyses • Sub-group 2 – Review, assess, and adjust protocol for Life Detection • Sub-group 3 – Review, assess, and adjust protocol for Biohazard Testing • Sub-group 4 – Environmental & health/monitoring and safety issues
5:30	Adjourn
6:00 pm	Reception

Day 2: Wednesday 6 June 2001

8:30 am	Day 1 Sub-groups report out in plenary session (30 min each) (*Sub-group Chairs*)
10:30	Plenary discussion 1 – Problems and issues associated with integrated protocol
12:00 pm	Day 2 Sub-group charters (*J. Rummel*)
12:30	Lunch
1:30	Sub-group deliberations • Sub-group 5 – Requirements of protocol for facilities, equipment • Sub-group 6 – Contingency planning for different protocol outcomes • Sub-group 7 – Personnel management considerations in protocol implementation • Sub-group 8 – Protocol implementation process and update concepts
5:30 pm	Adjourn

Day 3: Thursday 7 June 2001

8:30 am	Day 2 Sub-groups report out in plenary session (30 min each) (*Sub-group Chairs*)
10:30	Plenary discussion 2 – Research required needed to implement current protocol
11:30	Plenary discussion 3 – Review process/Open issues/Implementation status
12:30 pm	Lunch
1:30	Plenary discussion 4 – Research areas for protocol improvement in interim
2:30	Open items, discussion, assignments review
3:30 pm	Adjourn

APPENDIX D1
PARTICIPANTS' AREAS OF EXPERTISE

Name	Affiliation	Area(s) of Expertise
Acevedo, Sara E.	SETI Institute	(Workshop Planning Committee Member)
Allen, Carlton C.	NASA Johnson Space Center	Sample handling and curation; physical/Earth and planetary sciences.
Allton, Judith H.	NASA Johnson Space Center	Sample handling and curation; physical/Earth and planetary sciences.
Bada, Jeffrey L.	Professor, Marine Chemistry Scripps Inst. of Oceanography	Structure, Stability, and Evolution of Proteins; Life Detection
Battista, John	Dept. of Biological Sciences Louisiana State Univ.	Extremophiles *(D. radiodurans*, etc.)
Bibring, Jean-Pierre	IAS, France	Planetology; Sample handling; Curation facility
Bielitzki, Joseph	NASA Ames Research Center	Chief NASA Veterinary Officer
Cambon-Thomsen, Anne	Inserm U 518 Faculté de Médecine	Bioethics; President of French Committee on Planetary Protection
Chamberlain, Virginia	V.C. Chamberlain & Associates	Co-Chair, Sterilization Standards Committee, Association for the Advancement of Medical Instrumentation; Former director of FDA's sterilization compliance program
Clemett, Simon J.	Lockheed Martin Space Operations Houston TX	
Collins, Mary E.	Soil and Water Science Department University of Florida	Morphological, chemical, physical, and biological properties of hydric soils; subsurface variations of soil properties on karst areas.
Counil, Jean-Louis	Centre National d'Etudes Spatiale (CNES)	(Workshop Planning Committee Member)
Crissman, Harry A.	Los Alamos National Lab	Flow cytology and cytochemical Life Detection methods; Life Detection
Daly, Michael J.	Department of Pathology Uniformed Services University	Radiation resistant bacteria
Dawson, Sandra	NASA Jet Propulsion Laboratory	(Workshop Observer)
Debus, André	Centre National d'Etudes Spatiale (CNES)	Mars Sample Return Planetary Protection project manager
DeVincenzi, Donald	NASA Ames Research Center	(Workshop Planning Committee Member)
Edelson, C. Martin C.	Ames Laboratory U.S. Department of Energy	Environmental characterization of soils and other materials for DOE environmental assessments and statistical analyses. Laser-based methods for materials processing and characterization
Emmett, Edward	University of Pennsylvania School of Medicine	Medical monitoring; occupational health
Fishbein, William N.	Dept. of Environment and Toxicologic Pathology Armed Forces Institute of Pathology	Molecular toxicology; biochemical and molecular pathology; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis.
Foster, Virginia	Dept of Epidemiology & Biostatistics George Washington University	Biostatistics
Fox, George	Dept of Biology and Biochemstry University of Houston	Biochemstry

Name	Affiliation	Area(s) of Expertise
Fox, George	Dept of Biology and Biochemstry; University of Houston	Biochemstry
Friedmann, E. Imre	Florida State University	Microbiology in extreme environments; Life Detection
Fultz, Patricia	Department of Microbiology; University of Alabama at Birmingham	Microbiology
Gabriel, Dean W.	Department of Plant Pathology University of Florida	Molecular plant pathology; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis.
Garvin, James	NASA Headquarters	NASA Mars Program Scientist
Germaine, John T.	Geoenvironmental Research Group Massachusetts Institute of Technology	Development of lab and field instrumentation and testing of soils (e.g., Arctic silts); geotechnical engineering; Vice-Chairman, Committee D18 on Soil and Rock American Society for Testing and Materials (ASTM)
Giroir, Brett P.	Critical Care Medicine, Department of Pediatrics; University of Texas Southwestern Medical Center	Endotoxins in pharmacological studies
Grange, Jacques	Lab de Haute Securite P4 Jean Merieux	Responsible for the MERIEUX Biosfaety Level 4 facility; virology
Holland, Heinrich D.	Department of Earth and Planetary Sciences, Harvard University	Earth sciences; chemistry and evolution of the atmosphere and oceans
Hoyt, Diana	NASA Headquarters	NASA Space policy
Johnson, Dale W.	Desert Research Institute	Soil chemistry; physical/Earth and planetary sciences
Khan, Ali S.	Natl Center for Infectious Diseases Centers for Disease Control and Prevention	Biodefense; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis
Korwek, Edward	Law Offices, Hogan and Hartson	Environmental law and policy
Lambert, Joseph B.	Department of Chemistry; Northwestern University	Silicon polymer chemistry
Leonard, Debra G.B.	Dept. of Pathology & Laboratory Medicine; University of Pennsylvania	Molecular pathology of infectious diseases; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis.
Lindstrom, David	NASA Jet Propulsion Laboratory	(Workshop Observer)
Malling, Heinrick	National Institute of Environmental Health Sciences; National Institutes of Health	Biohazard Testing; Cellular and Molecular Genetic Mechanisms in Pathogenesis
Manhes, Gérard	Laboratoire de Géochimie et de Cosmochimie, France	Geochemistry and cosmochemistry
Maurel, Marie-Christine	Institut Jacques Monod	Microbiology; origin of life
McSweegan, Edward	Health Scientist Administrator, National Institutes of Health (NIAID/DMID)	Clinical research; Microbiology and infectious diseases
Mills, Aaron L.	University of Virginia; Dept of Environmental Sciences	Microbial Ecology
Mustin, Christian	Centre de Pédologie Biologique	Geologist and physicochemist; biochemical reactivity of microorganism-mineral interfaces.
Papanastassiou, Dimitri	NASA Jet Propulsion Laboratory	(Workshop Observer)
Pardee, Arthur B.	Dana Farber Cancer Institute; Harvard University	Molecular evolution; cell cycle control; cancer etiology.
Phillips, Mark	NASA Jet Propulsion Laboratory	(Workshop Observer)
Race, Margaret	SETI Institute	(Workshop Planning Committee Member)

Name	Affiliation	Area(s) of Expertise
Relman, David A.	Dept. Microbiology and Immunology; Stanford University	Microbial detection methods for unrecognized organisms; Life Detection
Richmond, Jonathan	Director, Office of Health and Safety; Centers for Disease Control and Prevention	Biosafety, emergent biohazard detection, and containment methods; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis.
Rummel, John	Planetary Protection Officer; NASA Headquarters	(Workshop Planning Committee Member)
Ryan, Margaret	DoD Center for Deployment Health Research; Naval Health Research Center	Deployment Health Research; Human performance
Scannon, Patrick J.	Chief Scientific and Medical OfficerXOMA (U.S.) LLC	Microbial pharmacology
Schad, Jack	NASA Headquarters	(Workshop Planning Committee Member)
Sogin, Mitchell L.	Biology and Evolution; Marine Biological Laboratory	Comparative molecular biology and evolution; Life Detection
Sourdive, David J.D.	Centre d'Etudes du Bouchet	Viral immunology, arena viruses; high sensitivity detection and identification of potentially hazardous microorganisms.
Stabekis, Pericles D.	Lockheed-Martin	(Workshop Planning Committee Member)
Stanbridge, Eric J.	Dept of Microbiology and Molecular Genetics; University of California, Irvine	Molecular detection of microorganisms in clinical settings; cancer etiology
Treiman, Alan H.	Lunar and Planetary Institute; Houston TX	Geology; physical/earth and planetary sciences
Vasil, Indra K.	Professor, Plant Cell and Molecular Biology; University of Florida	Plant tissue culture methods and biotechnology; Biohazard Testing; cellular and molecular genetic mechanisms in pathogenesis
Viso, Michel	Centré National d'Etudies Spatiale (CNES)	Radionuclides in biology, applied medical statistics, animal and comparative immunology, domestic animal nutrition
Voet, Donald	Department of Chemistry; University of Pennsylvania	Origins and evolution of life; physical and chemical analysis for Life Detection.
Wainwright, Norman	Senior Scientist, Molecular Biology; Marine Biological Laboratory	Comparative molecular biology and evolution; Life Detection

APPENDIX D2
WORKSHOP 4 PARTICIPANTS ROSTER

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APPENDIX E
OVERVIEW LECTURES

Summary of MSHP Workshops 1, 2, 2a, and 3

Margaret S. Race, SETI Institute

MSHP Workshop #4: Lecture 1
June 5, 2001 - Arlington, VA

Summary of MSHP Workshops 1-3 and Sterilization Workshop

Margaret S. Race
SETI Institute

Overview of Workshop Series

- WS 1: Bethesda, MD March 2000
  - Framing; Nature of Samples; Prelim. P-C, LD & BH tests
- WS 2: Bethesda, MD October 2000
  - ID & Prioritization of Candidate Tests; BH emphasis
- WS 2a: Arlington, VA November 2000
  - Sterilization
- WS 3: San Diego, CA March 2001
  - Unifying Properties of Life; Specific Methods
- WS 4: Arlington, VA June 2001
  - Comprehensive Draft Protocol Evaluation

WS 1: Bethesda, MD March 2000

Framing; Nature of Samples; Prelim. P-C, LD & BH tests

- SG1: Prelim. Sample Characteriz. Requirements (Maximize SCI. Info)
- SG 2 & 4: Sub-Samples; Prelim. P-C Analyses Flow Chart
  - 5-step process: removal; characterization; splitting; tests; release
  - emphasize non-destructive; minimal amount for tests
- SG 3: Sequence and Types of Tests (End to End); Results/Criteria
  - Biohazard Assessment and Clearance; Link with LD and PC tests
  - Carbon Assumption; emphasize Replicating Entities as well as Toxicity
  - Sequence of Questions & Strategy (structures; chemical;replication;adverse effects)
- SG 5: Prelim. Life Detection Tests
  - Gas, Fines, Pebbles, Cores (Non Destructive Scans vs Particle Sorting)
  - Combustion analyses & Mass Spec.– Complex molecules
  - Positive sorts- Microscopy, PCR, LAL, culture etc.
- SG 6: Prelim. Biohazard Tests- Both *in vitro* and *in vivo*
  - Combination of Cell and Tissue cultures; Established model systems
  - Varied responses (phenotypic; host gene expression; ecolog. microcosms

WS 2: Bethesda, MD October 2000

ID & Prioritization of Candidate Tests; BH emphasis

- **Life Detection Sub Group**
  - Non-Destructive (IR & Fluorescence Micro-spectroscopy; Light Microscopy; Head Gas analysis.; LD/MS & Laser Raman; 3D Tomography
  - Destructive (GC/MS; Extraction; Flow Cytometry; Cultures (terrestrial and 'martian' conditions);GC/MS; LC/MS; Enzyme Amplification techniques
- **Biohazard Sub Groups (2)**
  - Pathway to Decontainment; Multiple Models & Readouts; Passaging
  - Classes & Exs of Models: Verify containment materials; direct culture; cellular, small organisms,whole organisms, ecol. microcosms; monitor personnel etc. (plants, insects, microbes, mouse,human,ecosyst.)
- **Physical-Chemical Tests Sub Group**
  - Initial: Appearance, mass, major element comp; sample separation
  - Detailed: major/minor/trace element comp.; mineral comp; inorganic/organic carbon abundances

WS 2: (continued)

ID & Prioritization of Candidate Tests; BH emphasis

- **Molecular Biological Tests Sub Group**
  - No major role in martian LD per se- only false positives, background contamination levels & deleterious effects on terrestrial organisms. (DNA damage, gene express, altered gene expression etc.)
  - Actual tests TBD; Focus on Mars simulants as positive controls
- **Organism- & Cellular Based Tests Sub Group**
  - Outlined PPL designations (BSL plus cleanliness conditions)
  - Outlined important lab design concerns
  - Initial Testing (Human health emphasis): Human cell lines; mouse cells & microbial systems
  - Subsequent Tests- reduced containment level if earlier tests negative– (environmental emphasis) additional BH and geophysical testing

WS 2a: Arlington, VA November 2000 Sterilization

- Terrestrial Extremophiles as Models of how martian life might resist sterilization? Yes
- Worst Case Scenario for Sterilizing Martian Samples?
  - Infectious agent; remain in BSL-4 containment
- Best Methods & Procedures to Preserve Sample Integrity
  - No Ideal Method–Combination of heat and radiation methods
- If No Carbon or Polymers, Is sterilization required prior to distribution outside containment?
  - If no life or hazard detected, then OK to distribute w/o sterilization
- Can martian samples be safely distributed before LD, BH and other tests are completed?
  - Yes- decreasing levels of sterilization as data accumulated & interpreted.
- Can Martian Meteorites serves as models to test sterilization. procedures and effectiveness? Yes- but after test development on terrestrial analogs

WS 2a: Sterilization (continued)

- What are effective sterilization methods for martian samples?
  - 3A: Strictest level: Radiation/dry heat using virus based model (20X European Pharmacopoeia @ 120 degrees C)
  - 3B: Combination of Gamma ray or high-energy electron exposure plus simultaneous dry heat(Exposures & Temps. TBD-- likely >10Mrad and >95 degrees C)
    - Supplements to 3B:
      - Life based on Silicon Polymers
      - Sterilization by Ionizing Radiation

WS 3: San Diego, CA March 2001
Unifying Properties ; Specific Methods

- **Unifying Properties of Life**
  - Life = catalytic, genetic, replicates/evolves (measurable)
  - Avoid earth-centric approaches (yet method must recognize Earth life)
  - Focus on complexity, energy flow, oddities
  - Emphasize structural signs as first order task
  - Must recognize by multiple methods/signs, iterative approach
  - Inactive or past life treated as potentially active
  - Generalize carbon-centered methodology to other chem. species
- **Morphological Organization & Chemical Properties**
  - Assumptions about life based on Earth life
  - Possible Biosignatures of ET life: Microscopic morphology, structural chemistry; metabolism and bioenergetics; isotope signatures (distinctive fractionation);biologically induced geochem. Signatures
  - Recommended research areas: detection methods; enumerate cells and biomass; growth rates; metabolic activity, enzymatic activity

WS 3: San Diego, CA March 2001
Unifying Properties ; Specific Methods

- **Geochemical & Geophysical Properties** (for subsample selection)
  - Delineated Specific Properties and Criteria for all sample types:Gas; Head Space Gas; Bulk Fines; Rocks Fragments; Rock Cores; Soil Cores
- **Chemical Methods** (to detect low biomass or dormant martian life)
  - Detailed Flow charts & Protocol
  - (basic mineralogy; sub-micron morphology; inventory of biological elements; organic characterization)
  - Considered non-Carbon based life also
- **Cell Biology Methods**
  - Search for Complexity using strategic approach & multiple algorithms
  - Also Considered Non-Carbon Based Life
  - Specific Cell Biology Methods, Controls & Equipment Discussed

WS 3: San Diego, CA March 2001
Unifying Properties ; Specific Methods

- **What if ET Life is Detected?** (Plenary Discussion)
  - Science and Testing Issues (related to Protocol)
  - Facility and Technological Concerns (adequacy)
  - Policy and Administrative Concerns (risk communication; distribution; legal etc)

Report on the U.S. NRC Mars 2001 Sample Handling Study

John D. Rummel, NASA Headquarters

Planetary Protection

Report On:

The Quarantine and Certification of Martian Samples

US National Research Council
Space Studies Board
Committee on Planetary and Lunar Exploration

John A. Wood
Chair

May 2001

Membership: NRC Committee on Planetary and Lunar Exploration (COMPLEX)

Planetary Protection

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John M. Hayes, Woods Hole Oceanographic Institution
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Everett Shock, Washington University
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Charge to the Committee on Planetary and Lunar Exploration (COMPLEX)

Planetary Protection

- Focus on the requirements for a quarantine and biosafety certification facility for extraterrestrial samples, with the central question:
  - » What are the criteria that must be satisfied before samples can be released from the quarantine facility?
- Closely related issues include:
  - » What are the optimal techniques for isolating and handling planetary materials, determining their content of biota (if any), and carrying out basic geochemical characterization studies in the certification facility?
  - » How much capability for scientific analysis beyond that required for biosafety certification should be incorporated into the facility, and what principles should govern the utilization of this scientific capability?
  - » To what extent can valuable lessons be learned from the Apollo quarantine experience and from recent developments in the biotechnology and biomedical communities?

Detection of Life; Biohazards
Basic Assumptions and Basic Measurements

Planetary Protection

- Life [is] assumed to be carbon-based and microbial
- A series of tests can be envisioned that will provide evidence of viable or recently dead organisms, detect chemical fossils or probable biological molecules (biomarkers), and, at the same time, quantify contamination by terrestrial microbes and organic compounds.
- In order to avoid misinterpretation of "false positives," detection and identification of terrestrial contamination, both microbial and organic, will be crucially important.
- Measurement of total concentration of organic carbon in each martian subsample will provide an invaluable baseline. Samples in which organic carbon is below 10^{-12} gC/g are unlikely to contain microorganisms.

Detection of Life; Biohazards
Life Detection Experiments

Planetary Protection

- The actual detection of life, extant or recently alive, should be clearly distinguished from a search for the chemical traces left by life or for identifiable fossils.
- "....Essentially all our attention will be paid to organisms, largely ignoring viruses, viroids, prions, or other possible biohazards."
  - » First, the samples might contain biological materials that are similar to those found on Earth. In this case, all four categories of infectious agents are of concern, but then the means of sterilizing the samples are well understood.
  - » The second possibility is a life-form that is significantly different from terrestrial life. In this case, viruses and viroids could not replicate in terrestrial organisms, because of their reliance on a gene expression system different from that on the Earth.
  - » ....It is apt to be easier to detect organisms or their traces than to detect these other agents.

Detection of Life; Biohazards
Life Detection Experiments

Planetary Protection

- Cultivation studies will not provide high confidence that a sample is devoid of viable organisms. In spite of this, the intrinsic sensitivity of the method for organisms that do grow successfully, and the historical role of cultivation to detect contamination (by screening for common, easy-to-grow, terrestrial organisms) mean that culture-based studies are important and cannot be bypassed.
- Although having basic optical-microscope capabilities within the Quarantine Facility is important, electron microscopy and most other detailed examinations should be carried out on sterilized samples outside of the facility.
  - » Of course, this assumes the sterilization procedure would not destroy morphological evidence of life.
  - » There is a need to establish the effects of sterilization methods on microscopic morphological evidence of life.

Detection of Life; Biohazards
Life Detection Experiments

Planetary Protection

- Some of the most general and robust methods for detecting viable or recently dead life are based on detection either of the presence of specific molecules, or of chirality, or isotopic anomalies in molecules.
  - » In all of these cases, viability, or even cellular structure, is not required.
  - » Molecules whose presence can be taken as evidence of life are proteins, DNA, RNA, straight-chain fatty acids, and a variety of other lipids.
- A critical part of life detection in martian samples will be the ability to assess whether the source is extraterrestrial or terrestrial.

Detection of Life; Biohazards
Life Detection Experiments

Planetary Protection

- The search for evidence of former life, assumed to be carbon-based and microbial, seems certain to be a time-consuming, needle-in-a-haystack hunt.
  - » The first task should be to identify the haystack: to detect and profile the distribution of carbonaceous ("organic") matter in a representative subset of the total sample.
- The search for organic carbon should be accomplished early during sample processing, by surveying a sterilized representative subset of the sample outside of the Quarantine Facility.
  - » Such a survey will require use of extensive laboratory facilities that are stringently clean both of biological and organic terrestrial contaminants.
  - » To constitute strong evidence, evidence of earlier life must meet the tests of Indigenousness and Biogenicity.
- While the techniques described [here] can demonstrate or suggest the presence of life in martian samples, none of them can conclusively prove the *absence* of life.

Detection of Life; Biohazards Protocol

Planetary Protection

- Among other meanings of the word, a protocol is a written detailed plan formally specifying each step in a multistep scientific or medical procedure. In the context of the Mars Quarantine Facility, protocols must be written for procedures to:
  - » Sterilize and cleanse of organic contamination the Quarantine Facility, prior to introduction of the Mars samples;
  - » Place samples in the facility;
  - » Inventory and carry out preliminary analyses of the samples;
  - » Search for evidence of biological activity;
  - » Assess whether the samples contain biohazardous material;
  - » Sterilize aliquots of the samples in preparation for their removal from the facility;
  - » Remove samples from the facility; and
  - » Store samples within the facility.

Unequivocal Evidence of Life: COMPLEX's Recommendations

Planetary Protection

Unequivocal evidence of life would dictate a very elaborate plan of handling, curation, and study, which COMPLEX has not attempted to develop.

Recommendation:

- If unmistakable evidence of life as we know it is found in the Mars samples, they should be dedicated to biological studies. Studies of the biosignatures in them should be minimal until an optimal study plan has been developed, and an appropriate research facility set up and staffed. In the interim, no aliquots of the samples should be released from the confines of the Quarantine Facility unless warranted by ongoing biological studies, and the samples are sterilized.
  - » In this report the word life, when used in the context of martian life, should always be understood to mean "Life as we know it," to allow for the possibility of life forms distinctly outside our terrestrial experience.

Release of Samples from the Quarantine Facility: COMPLEX's Recommendations

Planetary Protection

- If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, release of unsterilized aliquots of the samples for study beyond the confines of the Quarantine Facility is justified.
- If the samples contain evidence of life, or if evidence of life is equivocal (*e.g.*, organic matter is present), aliquots that have been sterilized by heat and/or gamma radiation to levels more than adequate to kill any known terrestrial organism can be certified for release from the Quarantine Facility.
- If the samples contain evidence of life, or if evidence of life is equivocal, removal of unsterilized aliquots from the Quarantine Facility for transfer to approved containment facilities elsewhere should not be excluded, on the condition that containers and transfer procedures conform to protocols established by a panel of experts (*e.g.*, from the Center for Disease Control) in containment.

Equivocal Evidence of Life: COMPLEX's Recommendation

Planetary Protection

The discovery of life in the martian samples is unlikely....

Recommendation:

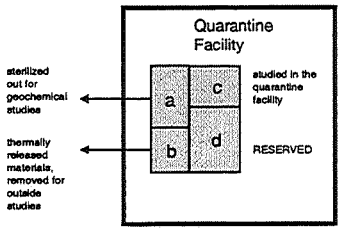
- In the likely event that initial examination of the Mars samples can neither prove nor definitively rule out evidence of life in them, plans should be in place to promptly sterilize aliquots of the samples and remove them from the Quarantine Facility for biological and geochemical studies in specialized laboratories elsewhere. This action should not be deferred pending some hypothetical future resolution of the question of whether the samples contain life or artifacts of life.

Detection of Life; Biohazards

Strategy for Quarantine & Distribution of Samples

Planetary Protection

- This strategy assumes the Mars samples are found to be neither manifestly barren of organic matter, nor obviously the bearers of live or recently dead organisms.



Aliquots
a – sterilized
b – crushed and organic compounds extracted
c – unsterilized
d – preserved for later study

Schematic Representation

Sterilization Research:

COMPLEX's Recommendations

Planetary Protection

Recommendations:

- It is important that a program of research be conducted to determine the efficacy of supercritical fluids and commonly used organic solvents in killing organisms. It is highly desirable to be able to remove solvent extracts from quarantine without the damage to dissolved biomarker compounds that would be caused by heat or ionizing radiation. Sterilization probably is systematically achieved by the supercritical fluids used in making extracts, but this needs to be verified before extracts can be removed from the Quarantine Facility.
- A program of research should be initiated to determine the effects on organic compounds in rocky matrices, and also on microscopic morphological evidence of life, of varying degrees of sterilization by heat and by gamma irradiation. This research should be started well in advance of the return of the Mars samples, so that sterilization protocols can be designed intelligently and so data obtained from analyses of sterilized samples can be interpreted with minimal ambiguity.

International Partnerships:
COMPLEX's Recommendation

Planetary Protection

The role of international partners in a sample return program should be carefully defined. The potentially sizeable contribution of another nation to the Mars program raises questions of how the earliest access and ultimate curation of the samples will be shared. *[It is beyond the scope of COMPLEX's charge to comment on the ultimate curation of the samples....]*

Recommendation:

- All samples in the initial collection returned from Mars should be placed in a Quarantine Facility in the United States, at least until the preliminary examination of the samples has been completed. Management and operation of the Quarantine Facility should be shared between the U.S. and major international partners who participated in the collection of martian samples.

Operations in the Quarantine Facility:
COMPLEX's Recommendation

Planetary Protection

COMPLEX considers that only the most basic operations should be conducted in the Quarantine Facility: unpacking, preliminary examination, baseline characterization, weighing, photography, splitting, repackaging, and storage. (In addition, certain life-detection studies which cannot be made on sterilized samples will have to be carried out in the Quarantine Facility.) To try and bring other scientific studies with bulky, complex instrumentation into the containment facility, along with the personnel that conduct the studies, would unacceptably increase the complexity, cost, and potential for failure of the facility.

Recommendation:

- The Quarantine Facility should be designed to the smallest and simplest possible scale consistent with its role as a biological containment and clean room facility. No scientific investigations should be carried out in the Quarantine Facility that can be executed on sterilized samples outside of the facility.

The Nature of the Quarantine Facility:
COMPLEX's Recommendation

Planetary Protection

Recommendation:

- A major obstacle to design of a Quarantine Facility is the problem of combining biological containment with clean room conditions. It is essential that work on the solution of this problem be started immediately, to include mockups of containment/clean room combinations whose efficacy can be tested, so the design of a Quarantine Facility can proceed.

Siting of the Quarantine Facility:
COMPLEX's Recommendation

Planetary Protection

COMPLEX considers that affiliation of the Quarantine Facility with an ongoing containment facility (USAMRIID, in Ft. Detrick, Md.; CDC, in Atlanta, Ga.; or the Medical Branch of the University of Texas at Galveston, where a BSL-4 facility is being constructed) is preferable to independent construction, for several reasons. These include:

1. Institutional support. A collaborative agreement with the host institution would mean the Mars Facility could draw on the latter for personnel, training, experience, security, and specialized utilities.
2. Economy. Sharing the resources named under 1. should effect a large economy in operation of the Mars Quarantine Facility.
3. Environmental Impact. Clearing an Environmental Impact Statement for a BSL-4 facility can take years. Ideally, the Mars Facility would operate under the Environmental Impact Statement of its host institution.

Recommendation:

- The Mars Quarantine Facility should be affiliated with an ongoing containment facility having BSL-4 capability, and should be physically part of it or proximate to it, but control of the Facility should be under the jurisdiction of NASA.

Time to Prepare the Quarantine Facility:
COMPLEX's Recommendation

Planetary Protection

"....the most important recommendation of this report:"

Recommendation:

- It is imperative that planning and construction of the Mars Quarantine Facility be begun at least 7 years in advance of the anticipated return of Mars samples. This responsibility cannot be deferred without compromising the quarantine and study of the Mars samples.

Lessons from the Apollo Experience:
COMPLEX's Recommendations

Planetary Protection

Recommendation:

- It is essential that the design for the Mars Quarantine Facility be kept as simple as possible, consistent with the facility's mission of protecting Earth's environment and the samples. Although it may be feasible to store the samples at low temperatures, an effort to try to maintain a Mars environment (temperature, pressure) during sample handling would complicate the design and operation of the facility to a very large degree, probably unnecessarily, and it should not be attempted for the first Mars sample return.

Recommendation:

- A continuing committee of senior biological and geochemical scientists that includes appropriate international representation should be formed, and charged with reviewing every step of the planning, construction, and employment of the Mars Quarantine (and initial sample handling) Facility. The committee should be formed during the earliest stages of planning of a Mars sample return mission. Members of the committee should also participate in the design of the spacecraft and those portions of the mission profile where biological contamination is a threat.

Other Recent Mars Planetary Protection Studies by the Space Studies Board

Planetary Protection

1992

- *Biological Contamination of Mars: Issues and Recommendations,* which reported advice to NASA on measures to protect Mars from contamination by Earth organisms, as well as overall policy guidance
  (Ken Nealson, Chair)

1997

- *Mars Sample Return: Issues and Recommendations*, which reported advice to NASA on Mars sample return missions
  (Ken Nealson, Chair).

SSB Recommendations on Forward Contamination of Mars (1992)

Planetary Protection

- Full "Viking-Level" sterilization is not required for missions to the martian surface, unless life-detection is a goal.
- New technologies to detect life are important and should be adopted to measure spacecraft bioload.
- "The Task Group strongly recommends that a sequence of unpiloted missions to Mars be undertaken well in advance of a piloted mission."
- "Missions carrying humans to Mars will contaminate the planet...The issues of forward and back contamination have societal, legal, and international implications. These implications are serious, and they deserve discussion and attention."

SSB Recommendations for Mars Sample Return (1997)

Planetary Protection

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

SSB Recommendations for Mars Sample Return (cont.)

Planetary Protection

- 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.
- 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.

SSB Recommendations for Mars Sample Return (cont.)

Planetary Protection

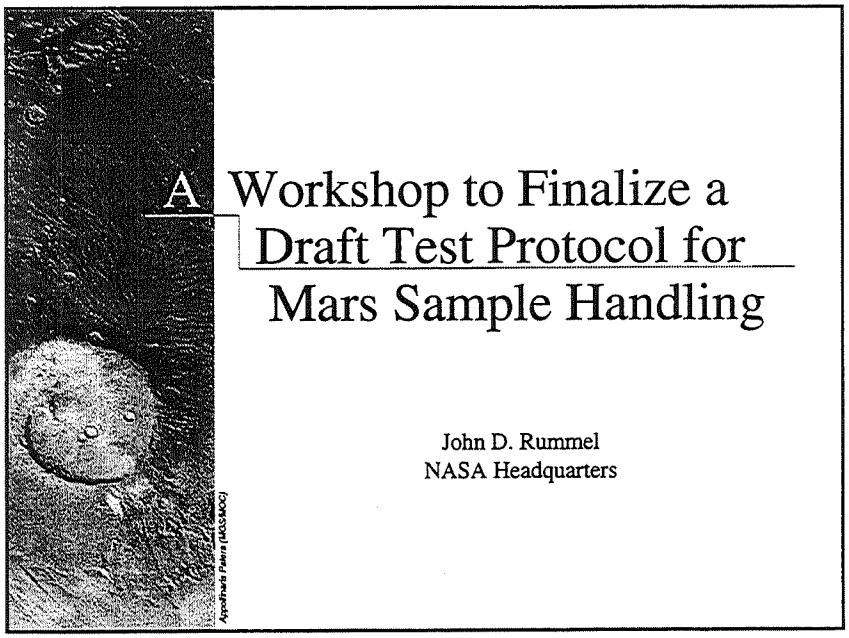
Technology Issues

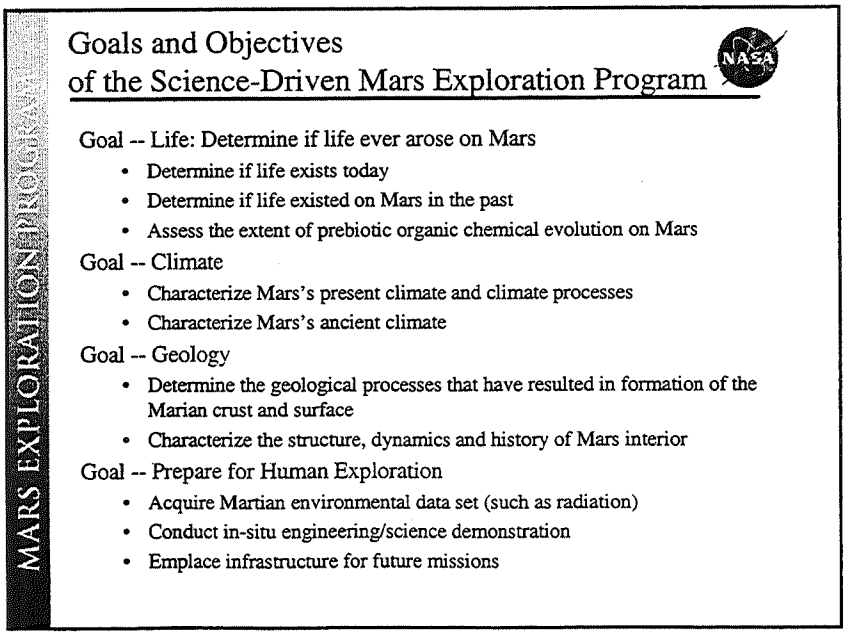
- Avoiding contamination of returned samples with organisms or organic material of terrestrial origin–

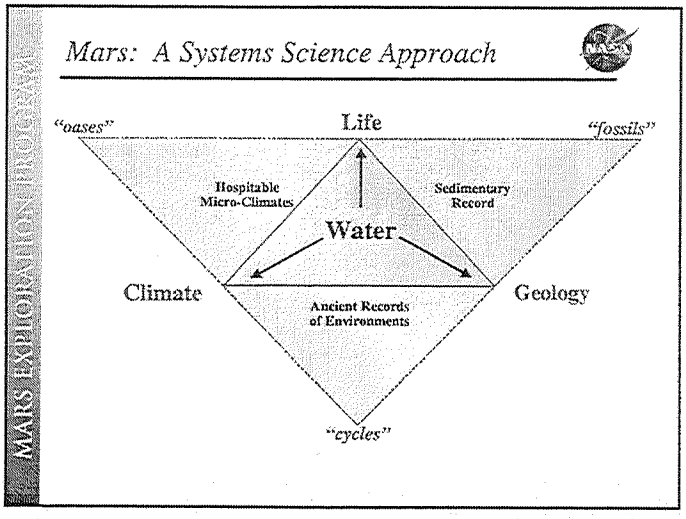
  "It will be important to stringently avoid the possibility that terrestrial organisms, their remains, or organic matter in general could inadvertently be incorporated into sample material returned from Mars. Contamination with terrestrial material would compromise the integrity of the sample by adding confusing background to potential discoveries related to extinct or extant life on Mars....Because the detection of life or evidence of prebiotic chemistry is a key objective of Mars exploration, considerable effort to avoid such contamination is justified."
- In-flight sterilization
- Sample handling and preservation
- Ensuring sample containment
- Avoiding return of uncontained martian material

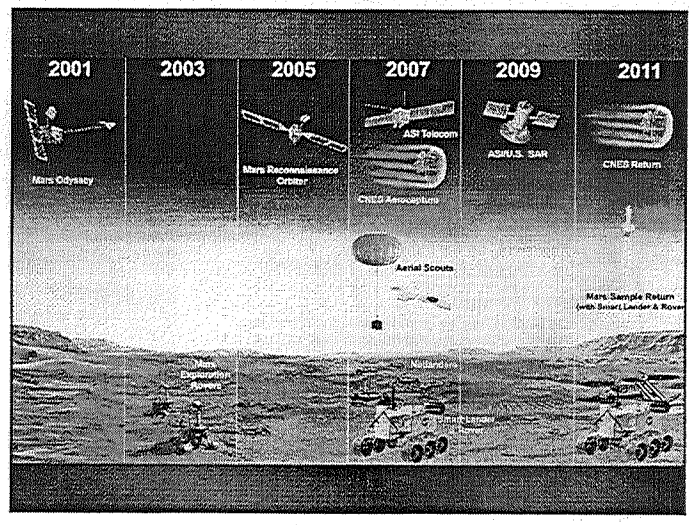
Overview of Draft Protocol and Workshop 4 Objectives & Sub-Groups

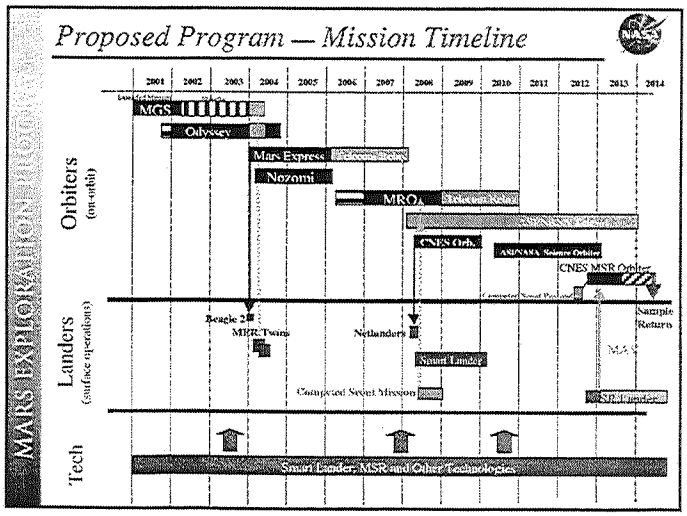
John D. Rummel, NASA Headquarters





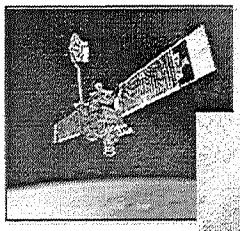

Mars: A Systems Science Approach
"oases"
Life
"fossils"
Hospitable Micro-Climates
Sedimentary Record
Water
Climate
Geology
Ancient Records of Environments
"cycles"
MARS EXPLORATION PROGRAM


2001
2003
2005
2007
2009
2011
Mars Odyssey
Mars Reconnaissance Orbiter
ASI Telecom
ASI/U.S. SAR
CNES Return
Aerial Scouts
Mars Sample Return

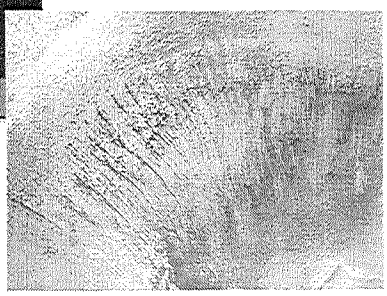

Proposed Program — Mission Timeline
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
MGS
Odyssey
Mars Express
Nozomi
MRO
CNES Orb.
CNES MSR Orbiter
Orbiters
(on-orbit)
Beagle 2
MER Twins
Netlanders
Smart Lander
Landers
(surface operation)
Sample Return
Tech
Smart Lander, MSR and Other Technologies
MARS EXPLORATION PROGRAM

MARS EXPLORATION PROGRAM

Mars Global Surveyor



Prime Mission: April '99 - Feb. '01
Extended Mission Just Begun!!



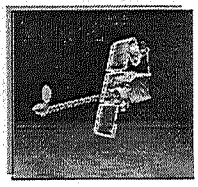
Major Instruments
Mars Orbiter Camera (MOC)
Laser Altimeter (MOLA)
Thermal Spectrometer (TES)

MARS EXPLORATION PROGRAM

2001 Mars Odyssey

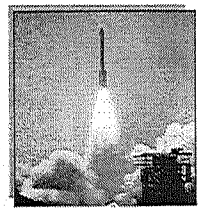
Mission Description

- ***Launch - April 2001 / Mars Orbit Insertion - October 24, 2001***
- ***Prime Mission - 76 days aerobraking, science mission through June 2004, relay mission through Oct. 2005***
- ***Science payload -***
  - *Thermal Emission Imaging System (THEMIS)*
  - *Gamma Ray Spectrometer (GRS)*
  - *Mars Radiation Environment Experiment (MARIE)*



Primary Objectives:

- THEMIS will map the mineralogy and morphology of the Martian surface using a high-resolution camera and a thermal infrared imaging spectrometer
- GRS will achieve global mapping of the elemental composition of the surface and determine the abundance of hydrogen in the shallow subsurface. GRS is a clone of the instrument lost with the Mars Observer mission.
- MARIE will describe aspects of the near-space radiation environment, especially the radiation risk to human explorers.
- Provide communications link for future Mars missions



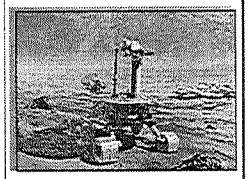
Successful Launch - April 7, 2001

MARS EXPLORATION PROGRAM

2003 Twin Mars Exploration Rovers

Mission Description

- ***Launch - May/June 2003 / Mars Landing - Jan/Feb 2004***
- ***Prime Mission - 90 days surface operations, until late April 2004; could be continue longer depending on health of the rovers.***
- ***"Athena" Science payload -***
  - *Panoramic Camera (Pancam)*
  - *Miniature Thermal Emission Spectrometer (Mini-TES)*
  - *Mössbauer Spectrometer*
  - *Alpha-Particle X-ray Spectrometer*
  - *Rock Abrasion Tool*
  - *Microscopic Imager*



Primary Objectives:

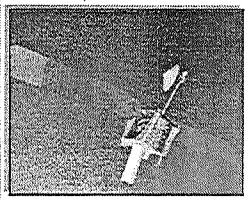
- Determine the aqueous, climatic, and geologic history of 2 sites on Mars where conditions may have been favorable to the preservation of evidence of pre-biotic or biotic processes.
- Identify hydrologic, hydrothermal, and other processes that have operated at each of the sites.
- Identify and investigate Martian rocks and soils that have the highest possible chance of preserving evidence of ancient environmental conditions associated with water and possible pre-biotic or biotic activity.
- Respond to other discoveries associated with rover-based surface exploration.

MARS EXPLORATION PROGRAM

2005 Mars Reconnaissance Orbiter

Mission Description

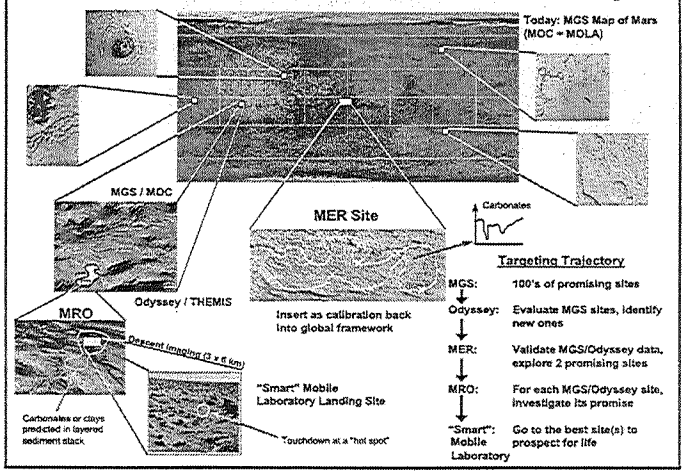
- *Launch - August 2005; enter Mars polar orbit*
- *Prime Mission - 3-5 years high resolution imaging and orbital characterization of Martian surface*
- *Science payloads under consideration*
  - *High resolution visible-near IR imaging spectroscopy (VNIRIS) (0.4 to 3 microns, 5 nm resolution, 50m/pixel)*
  - *High-resolution visible imaging (HRI) - (30-60 cm /pixel)*
  - *Infrared sounding and imaging of Martian atmosphere (MCO recovery)*
  - *Context imager*
  - *Other instruments under study*



Primary Objectives:

- Recover the Mars Climate Orbiter (MCO) MARCI and PMIRR investigation, emphasizing Mars volatiles (water) and climate history
- Search for mineralogic and morphologic evidence of water-related processes on a global basis
- Advance our understanding of the physical processes controlling the present transport, distribution and past evolution of water on Mars
- Conduct detailed study of regions of high scientific interest, including the Mars Global Surveyor discovery sites associated with "modern" water
- Characterize potential landing sites with regard to both scientific merit and landing safety
- 10 year extended mission telecommunication relay and navigation beacon

Scientific Reconnaissance as a Pathfinder to Where to Investigate



MARS EXPLORATION PROGRAM

Example Vision for 2012-2020

Respond to discoveries in previous decade.

Expand surface access to:

- Network science
- Near subsurface H_2O (to 200m)
- Deep Subsurface (>200m)
- High latitudes.

Multiple Mars Sample Return missions

Long-term virtual presence for public engagement

Planetary Protection Policy
(COSPAR / NASA)

"The conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized. In addition, the Earth must be protected from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from another planet or other extraterrestrial sources. Therefore, for certain space-mission/target-planet combinations, controls on organic and biological contamination carried by spacecraft shall be imposed in accordance with directives implementing this policy."

Recent Mars Planetary Protection Studies by the Space Studies Board

1992

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(Kenneth Nealson, Chair)

1997

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- Planetary protection measures adopted for the first sample return should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body

Planning for Sample Hazard Analysis (In Progress)

Protocol Development Workshops

- Plan: A series of workshops have been organized by NASA, with CNES participation, to assess the requirements for sample hazard testing and subsequent release, specify the tests necessary to show that a biological hazard is not present in the sample, and safeguard the samples against the various threats to its purity caused by the Earth's environment.
- For returned martian samples develop a requirements and recommended list of comprehensive tests, and their sequential order, that will be performed to fulfill the NRC recommendation that "rigorous analyses determine that the materials do not contain a biological hazard," taking into account the further recommendation that "returned samples should be considered potentially hazardous until they have been reasonably demonstrated to be nonhazardous."

Planning for Sample Hazard Analysis (In Progress)

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

Planning for Sample Hazard Analysis (In Progress)

Questions for Protocol Development Workshops

Consider:

- What criteria must be satisfied to demonstrate that the samples do not present a biohazard?
- What will constitute a representative sample to be tested?
- What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what physical/chemical analyses are required to complement biochemical or biological screening of sample material?
- Which analyses must be done within containment, and which can be accomplished using sterilized material outside of containment?
- What facility capabilities are required to complete the protocol? What is the minimum amount of time required to complete the protocol? How are these estimates likely to be affected by technologies brought to practice by 2012?

Questions/Issues: Final Workshop

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

Planning for Sample Hazard Analysis (In Progress)

Workshops, Finishing June 2001

- Protocol layout; Biohazard Determination; Sample Sterilization; Life Detection; Protocol Finalized
- Includes health and environmental monitoring and updating procedures

Post-Workshop Tasks

- Preparation of overall report and draft protocol details
- Review by Blue-Ribbon Scientific Advisory Panel and revisions
- Submit final document
- Endorsement by NAC / PPAC; Parallel review by foreign partners, etc.
- Dissemination of report to relevant audience(s) or Agencies for comment
- Approval by other Agencies, and availability for use in Mars Receiving Facility, etc.

Mars Sample Handling Oversight and Review Committee

Joshua Lederberg, Ph.D. *Co-chair*
Rockefeller University

Vacant

Members:
James R. Arnold, Ph.D.
University of California, San Diego
Purnell W. Choppin, M.D.
Howard Hughes Medical Institute
Dominique Dormont, M.D.
CEA - Service de Neurovirologie
Anthony S. Fauci, M.D.
National Institutes of Health
Nina V. Fedoroff, Ph.D.
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Gilbert S. Omenn, M.D., Ph.D.
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Washington University in St. Louis
Jean-Didier Vincent, Ph.D.
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Historical Consultant:
Steven J. Dick, Ph.D.
U.S. Naval Observatory
NASA Administrator's Liaison:
Kathie L. Olsen, Ph.D.
NASA Headquarters
Executive Secretary:
John D. Rummel, Ph.D.
Office of Space Science
NASA Headquarters

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 4 SUB-GROUP CHARTERS & MEMBERS

Sub-Group 1 Charter:

Review, assess, and adjust protocol for sample container processing, sample preparation, and physical/chemical analyses: Does the protocol adequately provide for planetary protection containment*, handling, and analysis requirements to protect the Earth, as well as for the requirements to ensure the scientific value of the sample? Can data about the sample be provided in a timely fashion to support the life-detection and biohazard determination steps of the protocol, as well as to support sample preservation and curation considerations? Which analyses need to be done in containment either within the primary containment facility or outside of containment using sealed containers? Which analyses can be done outside of containment on samples subjected to a sterilizing process, involving heat, radiation, etc., or a combination of these agents, to ensure they are safe for analyses outside of containment?

Treiman, Alan H. (US Co-Chairperson)
Counil, Jean-Louis (French Co-Chairperson)
Allen, Carl
Allton, Judith H.
Bibring, Jean-Pierre
Collins, Mary E.
DeVincenzi, Donald
Edelson, Martin C.
Garvin, James
Holland, Heinrich D.
Johnson, Dale W.
Manhes, Gérard
Mills, Aaron L.

Sub-Group 2 Charter:

Review, assess, and adjust protocol for Life Detection: Are data available from the first-tier physical/chemical analyses to support further analyses for Life Detection? Can the protocol be expected to yield evidence of living organisms within a martian sample? Can Earth organisms that might contaminate the sample be detected and identified as such? Can the protocol enable the detection of life-forms which are not based on Earth-biochemistry, but which have an active metabolism? Which analyses need to be done in containment either within the primary containment facility or outside of containment using sealed containers? Which analyses can be done outside of containment on samples subjected to to a sterilizing process, involving heat, radiation, etc., or a combination of these agents, to ensure they are safe for analyses outside of containment?

Relman, David A. (US Co-Chairperson)
Mustin, Christian (French Co-Chairperson)
Bada, Jeffrey L.
Clemett, Simon J.
Fox, George
Friedmann, E. Imre
Lambert, Joseph B.
Maurel, Marie-Christine
Sogin, Mitchell L.
Stabekis, Pericles D.
Voet, Donald
Wainwright, Norman

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 4
SUB-GROUP CHARTERS & MEMBERS (cont.)

Sub-Group 3 Charter:

Review, assess, and adjust protocol for Biohazard Testing: Are data available from the first-tier physical/chemical analyses to support the analyses for infectivity/biohazard (especially the presence of toxic materials)? Can the protocol be expected to yield sufficient evidence to rule-out any reasonable doubt over the absence of biohazard in the samples? Will the protocol allow for a broad-spectrum of challenges with the sample material that can reasonably be expected to show a response if the sample displays infectivity or a similar biohazard? Can the protocol results provide indications of the potential for chronic effects that should be assessed separately? Can Earth organisms that might contaminate the sample be detected and identified as such if a biohazard is detected? Which analyses need to be done in the primary containment facility, and which can/should be done outside of the primary containment facility using samples selected and shipped to another containment laboratory or kept in sealed containers?

Richmond, Jonathan (US Co-Chairperson)
Sourdive, David J.D. (French Co-Chairperson)
Battista, John
Bielitzki, Joseph
Chamberlain, Virginia
Fishbein, William N.
Foster, Virginia
Fultz, Patricia
Gabriel, Dean W.
Grange, Jacques
Khan, Ali S.
Malling, Heinrick
McSweegan, Edward
Pardee, Arthur B.
Schad, Jack
Stanbridge, Eric J.
Viso, Michel

Sub-Group 4 Charter:

Environmental and health/monitoring and safety issues: What sort of monitoring capabilities both within and outside of the containment area should be required to ensure the health and safety of the human workers in the primary receiving laboratory and any secondary facilities? What sort of capabilities should be required to ensure the adequacy of containment* and the safety of the environment outside the primary receiving laboratory? If no biohazard is found in the samples, and they contain non-biohazardous toxics or radioactive material, what measures to ensure safety should be required or recommended for those working with samples that are analyzed outside of containment — both in the case of samples subjected to a sterilizing process to ensure they are safe for analyses outside of containment, and in the case of samples that have been released for scientific study during or after sample recovery?

Leonard, Debra G.B. (US Co-Chairperson)
Cambon-Thomsen, Anne (French Co-Chairperson)
Crissman, Harry A.
Daly, Michael J.
Debus, André
Emmett, Edward
Giroir, Brett P.
Race, Margaret
Rummel, John
Ryan, Margaret
Scannon, Patrick J.
Vasil, Indra K.

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 4
SUB-GROUP CHARTERS & MEMBERS (cont.)

Sub-Group 5 Charter:

Requirements of protocol for facilities, equipment: What? Where? When? What if [a life-form or biohazard is detected]? What are the advantages/disadvantages of distributing the protocol activities among more than one containment facility? What factors should be considered in sizing the primary containment facility? What requirements should be met by secondary (PPL-α, BSL-4) facilities? Are there any other considerations that should be taken into account in providing a facility capability to enact the protocol?

Khan, Ali S. (US Co-Chairperson)
Bibring, Jean-Pierre (French Co-Chairperson)
Allen, Carl
Battista, John
Clemett, Simon J.
Collins, Mary E.
Counil, Jean-Louis
Fox, George
Friedmann, E. Imre
Garvin, James
Grange, Jacques
Johnson, Dale W.
Malling, Heinrick
Manhes, Gérard
McSweegan, Edward
Stabekis, Pericles D.

Sub-Group 6 Charter:

Contingency planning for different protocol outcomes: Given the various possible outcomes of the different protocol elements, what should be done at/in/around the containment facility(ies) if: 1) Absolutely no evidence of organic material is found in the sample? 2) The results from the protocol (esp. Life Detection/Biohazard Testing) are contradictory/ inconsistent? 3) A self-replicating entity or biomaterial(s), indicative of extant life is discovered within the sample materials? 4) That self-replicating entity cannot be shown to represent Earth-contamination.

Wainwright, Norman (US Co-Chairperson)
Maurel, Marie-Christine (French Co-Chairperson)
Bada, Jeffrey L.
Chamberlain, Virginia
Daly, Michael J.
Fishbein, William N.
Foster, Virginia
Gabriel, Dean W.
Holland, Heinrich D.
Lambert, Joseph B.
Mills, Aaron L.
Mustin, Christian
Relman, David A.
Schad, Jack
Stanbridge, Eric J.
Sourdive, David J.D.

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 4
SUB-GROUP CHARTERS & MEMBERS (cont.)

Sub-Group 7 Charter:

Personnel management considerations in protocol implementation: What are the requirements for personnel to complete the protocol, as written? When do personnel need to be hired and trained? What considerations can be given to the qualifications of required personnel, and the selection process by which personnel are chosen to: 1) Conduct the various elements of the protocol? 2) Provide for the appropriate biosafety considerations and containment at the primary and any secondary facilities? and, 3) Conduct any required analyses that are of scientific interest or are also necessary to support preservation and curation of the martian samples (e.g., time, processing-dependent studies)? What external advice/oversight capabilities should be available to support the execution of the sample-handling protocol (e.g., to ensure that the protocol is executed according to plan, and that if modifications are necessary they are approved and documented)?

Vasil, Indra K. (US Co-Chairperson)
Viso, Michel (French Co-Chairperson)
Allton, Judith H.
Crissman, Harry A.
Debus, André
Edelson, Martin C.
Emmett, Edward
Giroir, Brett P.
Leonard, Debra G.B.
Richmond, Jonathan
Ryan, Margaret
Voet, Donald

Sub-Group 8 Charter:

Protocol implementation process and update concepts: How should the final review and modification of this protocol be conducted? What steps should be taken in gaining approval of the final Draft Protocol by national and international bodies important to its acceptance/implementation? How should the Draft Protocol be maintained and after its initial approval and promulgation? What steps should be available to the protocol implementers to provide for proposed changes in the details and/or framework of the final Draft Protocol once it has received initial approval? What process should be followed to reaffirm acceptance/approval of the final protocol to be used for the actual samples? What regulatory steps (if any) should be taken to certify the samples are safe for release from containment after the protocol is completed?

Bielitzki, Joseph (US Co-Chairperson)
Cambon-Thomsen, Anne (French Co-Chairperson)
DeVincenzi, Donald
Fultz, Patricia
Korwek, Edward
Pardee, Arthur B.
Race, Margaret
Rummel, John
Scannon, Patrick J.
Sogin, Mitchell L.
Treiman, Alan H.

"Floaters" (Attendees who will be observing the Sub-Group deliberations):

Acevedo, Sara E.
Briggs, Geoffrey
Dawson, Sandy
Hoyt, Diana
Lindstrom, David
Papanastassiou, Dimitri A.
Phillips, Mark

APPENDIX F
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APPENDIX G
GLOSSARY OF TERMS AND ACRONYMS

ALH	Alan Hills (Antarctica)
ATP	Adenosine Triphosphate
BFP	Blue Fluorescent Protein
BSL	Biosafety Level
CAPTEM	Curation and Analysis Planning Team for Extraterrestrial Materials
CCNE	"Comité Consultatif National d'Ethique pour les Sciences de la Vie et de la Santé", CCNE (French)
CDC	Centers for Disease Control and Prevention (U.S.)
'cleanliness'	Freedom from biological or chemical contamination
CNES	Centre National d'Etudes Spatiale (French)
CNRS	Centre National de la Recherche Scientifique (French)
COMPLEX	Committee on Planetary and Lunar Exploration
'coupons'	Small, regular samples of solid laboratory materials such as plastic
D_{37}	The average radiation dose required to inactivate a live or infectious particle
DNA	Deoxyribonucleic Acid
Eh	Oxidation Potential
EPST	Etablissements Publics à Caractère Scientifique (French)
EVT	Experiment Verification Test
GC/MS	Gas Chromatograph/Mass Spectrometer
GFP	Green Fluorescent Protein
HEPA	High Efficiency Particulate Air (filter)
IACUC	Institutional Animal Care and Use Committee
IBSC	Institutional Bio-Safety Committee
ICBC	Interagency Committee on Back Contamination
i.c.	Intracranially
i.p.	Intraperitoneally
IR	Infrared
Knockout mouse	A mouse that is genetically engineered (both alleles of a critically targeted gene are replaced by an inactive allele using homologous recombination) to produce a particular designer alteration whereby a specifically targeted gene becomes inactivated (or "knocked-out")
LAL	*Limulus* Amebocyte Lysate
LC/MS	Liquid Chromatograph/Mass Spectrometer
LD/BH	Life Detection/Biohazard (Testing)
LD/MS	Laser Desorption Mass Spectroscopy
Mrads	Megarads

MS	Mass Spectroscopy
MSHARP	Mars Sample Handling and Requirements Panel (U.S.)
MSHP	Mars Sample Handling Protocol
MSR	Mars Sample Return
NAS	National Academy of Science (U.S.)
NASA	National Aeronautics and Space Administration (U.S.)
NASA-CP	NASA Conference Proceedings
Nd:YAG	Neodymium-doped:Yttrium Aluminum Garnet (LASER)
Nude mouse	A mouse which lacks a thymus and, therefore, cannot generate mature T lymphocytes to mount most types of immune responses
NIH	National Institutes of Health (U.S.)
NRC	National Research Council (U.S.)
PAH	Polycyclic Aromatic Hydrocarbon
'passaging'	A sub-culturing technique
P/C	Physical and Chemical (Testing)
PCR	Polymerase Chain Reaction
pH	Measure of hydrogen ion concentration (acidity)
PP	Planetary Protection
PPC	Planetary Protection Committee
PPAC	Planetary Protection Advisory Committee
PPL	Planetary Protection Level
PWDP	Penultimate Working Draft Protocol
'readout'	A measure of potential biohazard effect
'riffle splitter'	A mechanical separation device used for geological samples
RNA	Ribonucleic Acid
'rocklets'	Millimeter-sized rock fragments
SCID	Severely Compromised Immunodeficient (non-human cells, usually mouse)
SCID-Hu	Severely Compromised Immunodeficient (Human cells)
'simulant'	Analogue
SRC	Sample Return Canister
SRF	Sample Receiving Facility
SSB	Space Studies Board
TBC	To Be Confirmed
TBD	To Be Determined
TEM	Transmission Electron Microscopy
TOC	Total Organic Carbon
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USDA	U.S. Department of Agriculture
UV	Ultraviolet

WHO	World Health Organization
'witness plates'	Controls for forward contamination; used to monitor for bioload on spacecraft
XRD	X-ray Diffraction
XRF	X-ray Fluorescence (Spectrometer)

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14. ABSTRACT

This document is the proceedings of the final workshop in the Mars Sample Handling Protocol Workshop Series; Workshop 4 was convened June 2001 in Arlington, Virginia. The Workshop Series was designed by NASA's Planetary Protection Officer to develop a comprehensive protocol by which returned martian sample materials could be assessed for the presence of any biological hazard(s) while safeguarding the purity of the samples from possible terrestrial contamination. A Working Draft of the Protocol is included in the report. The reference number for the Final Draft Protocol is NASA/CP-2002-211842 and for the proceedings from Workshops 1, 2, 2a, and 3 are: NASA/CP-2000-20964, NASA/CP-2001-210923, NASA/CP-2001-210924, NASA/CP-2001-211388.

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