Planning for the Analytic Environment to Conduct Life Detection Experiments on Samples Returned from Mars: Observations and Issues

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Introduction: If samples were to be brought from Mars to Earth by a robotic sample return mission, the samples would initially reside in a specialized facility capable of highly reliable biocontainment. At a minimum samples would need to be sterilized or assessed for any potential biohazard [1]. In an alternate model, a significant capability for scientific research might be designed into the facility so that a variety of analyses could be accomplished while keeping the samples in biocontainment. What does this imply for researchers who might aspire to study samples returned from Mars? Further, what would it mean to the planetary protection community to consider alternate approaches to meeting the requirements for biohazard assessment as set forth in international policy?

Definitions: Biocontaiment laboratories are classified in biosafety levels (BSL) 1 to 4. BSL-1 laboratories are suitable for studies involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment [2]. At the other extreme, BSL-4 laboratories are designed to contain and allow the manipulation of dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, and agents which cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as, Marburg virus, Ebola virus, Lassa fever, smallpox, and various other hemorrhagic diseases. BSL-4 laboratories are designed within larger facilities with lower levels of containment. In addition to the designation of safety levels for laboratories as a whole, there is also a classification scheme for cabinets that are used to conduct experiments on different types of pathogens [3]. Biosafety Cabinets (BSC) of Class II and above use airflow and HEPA filtration to minimize aerosol contamination of the surrounding environment. Class III enclosures are gas-tight, and all materials enter and leave through a dunk tank or double-door autoclave. Gloves attached to the front prevent direct contact with hazardous materials. These custom-built cabinets often attached to each other, and the lab equipment installed inside is usually custom-built as well. In some BSL-4 laboratories, the primary barrier for containment is the BSC. The suit covering the personnel only improves the containment, and is considered a secondary barrier.

Formal advice to NASA and others who would undertake sample return from Mars has been that the highest containment standard—Biosafety Level 4—would be required for study of unsterilized Martian samples [1]. This applies equally to a life detection and biohazard assessment that would be a mandatory prerequisite for ultimate release of samples from biocontainment. In addition to the highest levels of containment, returned Martian samples would be subject to extremely stringent levels of contamination control to protect the scientific integrity of the samples. This is one of the unique aspects of a Mars Sample Receiving Facility (SRF)—the combination of high level biocontainment and combined with contamination control. NASA scientists and engineers have visited a number clean room and BSL-4 laboratories to understand the specific designs and choices of laboratories that might guide the design and development of a Mars SRF. All visited laboratories share some common strategies, but they incorporate a number of differences depending upon the particular facility's charter.

On September 27, 2011, a group of scientists representing NASA's Mars Exploration Program visited the National Biodefense And Countermeasures Center (NBACC) to understand some of the key design challenges. The Department of Homeland Security built the NBACC laboratories to enhance its capabilities to protect the American public from bioterrorism. This research facility is located within the National Interagency Biodefense Campus at Ft. Detrick, Maryland. More information regarding NBACC can be found at http://www.bnbi.org/.

Observations of Existing Biosafety Labs:

• *Significant experience in working with hazardous materials.* While the notion of working with pathogens and biosafety levels may be new for many at NASA, working in these environments (BSL-2, 3, 4) is relatively commonplace, with an entire industry devoted to the design, construction, and operation of these facilities. NASA should continue to consult with NBACC and others who operate BSL-4 laboratories, using them as a resource to avoid duplication of research for Mars Returned Sample Handling (MRSH) and sample handling protocols. For example, the safe transport of samples within and between BSL-4 laboratories – a recognized requirement for Mars

Sample Return – is well established in the biosafety community.

• *Modular designs.* Construction details of the NBACC labs of particular interest to the NASA science team included access chambers and a modular design. For example, NBACC's facility has substantial access chambers adjacent to the laboratory spaces for moving large equipment in and out of the facility to address the issues of equipment maintenance and biocontainment. Also, the NBACC design did not attempt to predict all future uses, but rather adopted a modular approach in which a given space could be modified to enable a particular task to be completed. This approach may be useful for NASA since the particular measurements (and hence instruments, preparation procedures, and curation restrictions) would likely not be understood until the samples are examined in some detail. These sorts of design choices would be made during the construction phase of a Mars SRF.

• *Laboratory Sterilization.* The NBACC containment labs are clean from the point of view of live biology, since a significant portion of their purpose is to search for trace organisms. The labs are periodically sterilized by introduction of vaporized hydrogen peroxide (VHP) or formaldehyde gas. Equipment is passed through an autoclave on its way in and out of some laboratories. Typically, BSL-4 labs can tolerate higher background of dead organisms and organic molecules than will be acceptable for MRSH. Furthermore, for Mars Returned Sample Handling, sterilization procedures may need to be re-examined. For example, there are organisms on Earth that can survive autoclaving. Also, the Earth-based standard techniques for whole room decontamination (*e.g.*, vaporized hydrogen peroxide or formaldehyde) could be a concern because it is unknown whether putative Martian organisms can withstand or perhaps even metabolize hydrogen peroxide. [4] While 'extreme' organisms are unlikely to be pathogenic, if sterilization is required, it would need to be definitive. This may be at odds with equipment requirements as well as organic compound detection, etc., which are already recognized issues.

• *Cleaning.* Another take-home message is the difficulty of cleaning a BSL-4 facility to the class 100 cleanroom conditions necessary to conduct inorganic analysis of the type commonly applied to planetary materials. One possibility that could be explored, is building a cleanroom space within the BSL-4 facility for this type of analytical procedure. The combination of stringent cleanroom and biosafety reqirements in a single facility is not common, and would require extensive development, testing and certification. Alternatively, inorganic analyses could be completed on samples outside the BSL-4 facility, if suitable sample sterilization protocols could be developed.

• *Instrumentation needs.* The visiting science team could not envision how it would be possible to clean these labs to the point that scientists could conduct experiments regarding trace evidence for the signs of unknown life with Martian samples on open benches. The required kind of environment would only be credibly created inside biosafety cabinets. Therefore, there is an as-yet undefined relationship between the instrumentation (that will do the life detection), the clean environment, and the containment barrier. A significant program of research, development and certification will be required to meet these key science requirements.

• *Stand-alone vs. Add-On.* After the required planetary protection testing has been executed, biocontainment may not be necessary. As a result, NASA would not wish to have built a significant facility that would be abandoned after several years of use. As a result, NASA should consider building a facility that might have capabilities beyond bio-containment (*e.g.* curation capabilities for future study).

Conclusions: While the NBACC laboratories and other BSL-4 labs satisfy some of the requirements of a sample receiving facility, the primary mission of BSL-4 labs and their goals are inherently different. Addintionally, NBACC also deals primarily with fairly well-known organisms. NASA needs to scrutinize carefully the advice and expertise from existing biosafety laboratories, and be cognizant that there will be issues during sample return evaluation and curation that will require independent thinking and solutions.

It is clearly necessary to begin the planning for an SRF early in the Mars Sample Return Campaign. Previous studies conducted by the National Research Council [5] recommend that planning for an SRF begin at least seven years prior to the return of the Martian samples.

References: [1] Rummel et al. (2002) *NASA/CP-2002-211842*. [2] Center for Disease Control (1997); [3] U.S. Centers for Disease Control and Prevention, U.S. National Institutes of Health (2000). U.S. Centers for Disease Control and Prevention. [4] Kashefi and Lovley (2003) *Science* 301, 934; Takai et al. (2008) *PNAS* 105:31,10949–10954; Schulze-Makuch et al. (2008) *Astrobiology* 8:2, 205-214. [5] National Research Council (2002) *Space Studies Board Committee on Planetary and Lunar Exploration (COMPLEX)*, National Research Council, National Academy Press, Washington D.C.