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EURO-CARES
A PLAN FOR EUROPEAN CURATION
OF RETURNED EXTRATERRESTRIAL SAMPLES

PLANETARY PROTECTION

PRELIMINARY REQUIREMENTS:

REVIEW AND CURRENT STATE-OF-ART

WP 1 Task 1.2 D1.2

Issue 1 Rev. 1

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1. Introduction

In this study a review of technology and science requirements in Planetary Protection and contamination control is conducted. Literature publications dealing with Planetary Protection issues for a curation facility's needs for future sample return missions are summarised in this report. This document addresses the issues identifying how Planetary Protection impacts on system technologies and scientific measurements with a final aim to prioritise outstanding any technology shortfalls.

This report addresses Planetary Protection specifically in areas related to the design of curation facility for samples returned from Asteroids, Comets, moons and Mars. Specifically, the major needs are related to:

- Sample containment;
- Transportation;
- Receiving facility;
- Sample handling;
- Preliminary examination;
- Sample extraction;
- Biohazard testing;
- Sample storage;
- Sample delivery to laboratories.

2. Planetary Protection Overview

Samples returned from asteroids, moon and Mars are subject to satisfy planetary protection regulation. The legal aspects of planetary protection are agreed by all space agencies and they are the guiding principle in the design of space mission to protect solar system bodies from contamination by Earth life forms, and protecting Earth from possible life forms that may be returned from other solar system bodies. The Committee on Space Research (COSPAR) has the mandate from the United Nations to maintain and promulgate the planetary protection policy, both as an international standard on procedures to avoid organic-constituent and biological contamination in space exploration, and to provide accepted guidelines to guide compliance with the wording of UN Space Treaty and other relevant international agreements.

Planetary protection is essential to preserve our ability to study astrobiologically interesting planets and moons of our Solar System. The contamination of celestial bodies by Earth organisms and organics needs to be avoided because it could lead to false-positive results. This would reduce any possibility of detecting native life on that body, if it exists, and a false positive result would also place unnecessarily stringent planetary protection requirements on future missions where these would be in place under the false assumption that life had been found (forward contamination prevention). The second aspect of planetary protection aims to protect the Earth's biosphere from extra-terrestrial agents, which might be harmful if released into the Earth environment (backward planetary protection prevention).

COSPAR defined five planetary protection categories with subcategories dependent on the target of the mission and the type of mission (fly-by, orbiter or lander). Category I missions do not have planetary protection requirements, e.g. for missions to undifferentiated, metamorphosed asteroids or Io. Category V missions include the most stringent planetary protection requirements. All missions which will return extra-terrestrial samples to the Earth for further analysis belong to category V. Dependent on the origin of the extra-terrestrial material a category V mission can be an unrestricted Earth return mission (e.g. with samples from the Moon) or restricted Earth return mission (e.g. with

samples from Mars or Europa). For what concerns our work the categories that significantly affect the sample curation solutions for the design and the operational protocols for sample handling are category IV and V. Specifically:

- *Category IV: Lander or probe missions to locations of significant interest for chemical evolution and/or origin of life, and with a significant chance that contamination could compromise investigations (Mars, Europa, Enceladus).*
- *Category V:*
    - Unrestricted: samples from locations judged by scientific opinion to have no indigenous lifeforms. No special requirements (Moon).
    - Restricted: (where scientific opinion is unsure) the requirements include: absolute prohibition of destructive impact upon return, containment of all returned hardware which directly contacted the target body, and containment of any unsterilized sample returned to Earth (Mars, Europa).

As a consequence, future sample return missions from Mars raise serious questions about biological and organic contamination measures that should be taken to protect samples from Earth-sourced biological and organic contamination but also to protect Earth from possible living organism found on the returned samples.

In this report we will review the state of art of molecular and biological contamination. Techniques for life detection will be showed and, in particular, major studies on curation facility for samples returned from Mars will be addressed.

3. Contamination issues

Contamination prevention must maintain the samples in their pristine status state for long-term storage (years). Contamination prevention strictly depends on the nature of returned samples. In a first step analysis, the samples returned by missions visiting asteroids, the Moon and Mars will be likely to be heterogeneous regolith with the following principal chemical characteristics:

- *Inorganic compounds:* ferromagnesian silicates, aluminosilicates, Fe and Cr oxides, phosphates, metals, sulphides, carbides, nitrides, and hydrated silicates (e.g. clays).
- *Organic compounds:* soluble carbonaceous and insoluble kerogenous-like compounds, graphite, aliphatic and aromatic hydrocarbons, heterocyclic compounds, amines and amides, alcohols, carbohydrates, biomolecules and, possibly, simple life forms.

It is not excluded that gaseous species and liquids will be also delivered within the re-entry capsule.

Contamination is defined as molecular, liquid and particulate material that could be adsorbed in a certain amount and alter the sample characteristics or degrade the returned samples. Molecular contamination is defined as any gaseous chemical substances. Liquid contamination is any compound (water, organic, metal) with no fixed shape able to flow easily at room temperature and pressure. Particulate contamination is any inorganic, organic and biological tiny (0.01 – 100 mm size) solid particles. Thus, any transfer of chemicals, liquids or particulates of terrestrial origin (environmental, human, processing, facility, equipment and working activity) to the samples has to be minimized.

The contamination prevention must be regularly satisfied during all the following operational tasks inside the Curation facility:

- Earth Re-entry Capsule (ERC) handling;
- Sample handling;
- ERC storage;
- Planetary protection activities (life detection and biohazard assessment);
- Sample storage;

- Sample characterization;
- Sample delivery to external laboratories and retrieving;
- Maintenance of curation facility.

3.1. Contaminants

A partial list of common contaminants that can affect Curation Facility environments are reported in Table 1. Many of these contaminants are generated from four basic sources: the facility, people, tools, and product generated (Brucato et al. 2012).

All areas adjacent to the cleanroom are generally to be less clean than the cleanroom itself and material airlock and clothing-change area will be contaminated by the activities going on in these areas. It also contains contamination dispersed into it from other sources, such as people, machines and instruments. The floor, walls, ceiling and other surfaces in the cleanroom are examples of surface sources, their contamination being mostly derived from personnel touching them, their contamination depositing from air. Cleanroom clothing, gloves and masks are other surfaces that are contaminated either by the people wearing them or by other cleanroom surfaces. Personnel within the cleanroom can disperse contamination from the skin, mouth and clothing. This contamination can be transferred to the sample through the air, or by contact with their hands or clothing. Machines are another source, as they can generate contamination by the movement of their constituent parts, or by generation by thermal, electrical or other means. Raw materials, sample containers and packaging that are brought in, or piped into the cleanroom, may be contaminated and should be considered as sources.

Table 1 Common environmental contaminants

Source	Contaminant	Type
Facility	Surface coatings: walls, floors and roofs	Particulate & liquids
	Surface desorbed water	Liquids
	Building materials	Particulate
	Air conditioning	Particulate, liquidss
	Room air	Particulate, molecular
	Spills and leaks	Liquid, molecular
	Air filters	Particulate
	Packing	Plasticizers and liquidss
	Containers	Particulate, flakes
People	Skin	Biological (cells), flakes
	Skin fat	Liquids
	Cosmetics	Molecular, particulate
	Spittle	Liquids
	Clothing fibers	Particulate
	Particles in hair or clothes	Particulate
	Hair	Biological thread
	Bacteria, fungi and viruses	Biological
	Water	Molecular, liquids
	Organics	Molecular
	Secondary microorganism products	Molecular, biofilm
Tools	Friction and wear	Particulate
	Lubricants and emissions	Molecular, liquids
	Vibrations	Particulate, thread
	Brooms and mops	Solids
	Spatters	Liquids, solid film
	Cleaning chemicals	Molecular, liquids
	Plasticizers	Molecular (outgases)
	Adhesive plates	Molecular
	Machine oils	Liquids
Product generated	Teflon	Flakes, molecular
	Quartz	Flakes
	Aluminium	Particulate, molecular
	Gold	Particulate, molecular
	Stainless steel	Particulate, molecular
	Coating metal	Flakes

Known contaminants will be monitored periodically both by passive witness plates mounted in different places of the facility and by active monitoring instruments. Concerning unknown contaminants, tests and analyses should be performed periodically (e.g. outgassing rates as a function of time, chemical composition of outgassing products, condensation rates or degradation as result of radiation). The results of these tests and analyses will be used to calculate expected contamination levels and their subsequent effects on returned sample status if other relevant parameters are known and engineering control need to be developed to remove or minimize to hazardous biological materials.

4. Mars Sample Containments

Samples returned from Mars can pose a risk to the Earth's biosphere if they contain potentially hazardous agents; therefore breaking the chain of contact between the Martian and Earth environments is a necessity when space mission is designed. For Mars sample return missions (MSR) ESA Planetary Protection Requirements (ESA Planetary Protection Requirements_ESSB-ST-U-001, 2012) requests that the probability that a single unsterilized particle from Mars of ≥ 0.2 mm in diameter is released into the terrestrial biosphere shall be ≤ $1x10^{-6}$. An ESF-ESSC Study Group on MSR Planetary Protection Requirements was asked by ESA to recommend the level of assurance for the exclusion of an unintended release of a potential Mars life form into the Earth's biosphere for a MSR mission taking the newest state of knowledge in the areas of biology, technology, risk assessment and risk perception into consideration. The findings and recommendations of this study were published in 2012 (Ammann et al., 2012). The study group came to the following recommendation: The probability that a single unsterilized particle of 0,01 mm diameter or greater is released into the Earth's environment shall be less than 10^{-6}. The reduced size of the particle within the requirement, 10nm in comparison to ≥20nm, provides a more stringent test for the containment of the facility and its incorporated technologies to conform to the sterilisation limit. This would need to be one of the main factors considered during the design of the facility.

5. Spacecraft Sterilization

The interpretation of sample analyses once studied on Earth is critical because science-driven organic contamination control is inextricably coupled to Planetary Protection measures. While some techniques to control the chemical cleanliness of surfaces are also effective in reducing the bioburden, others may work against the science goals. An example is the use of alcohols by spacecraft engineers for cleaning surfaces. Ethanol or isopropanol are, in fact, used by biologists to inactivate the majority of bacteria. However, these alcohols cannot be used for sterilization because they are not effective against bacterial endospores and some other robust microorganisms. Alternative techniques for sterilisation and decontamination are used in the microbiological industry, such as gaseous disinfection where a chemical is vaporised into a sealed enclosure and acts on the organisms present on the surfaces. There are a number of gaseous decontamination technologies commercially available, and standards are available for their use (ECSS-Q-ST-70-56C: Vapour phase bioburden reduction for flight hardware). But whilst these techniques will inactivate the microorganisms present on a surface, the remaining whole organism or components of it would still be present on the surface and present as chemical/biological contamination which could lead to issues in interpretation of any test undertaken. Of the many Planetary Protection technology initiatives that will be explored to design the curation facility, the cleaning and contamination control measures are most directly linked to science requirements.

The exposure of spacecraft materials, components, subsystems and also of whole spacecraft, as previously undertaken for the Viking landers, to dry heat microbial reduction (DHMR) in a standardised process was the first bioburden reduction technique officially accepted by space agencies. As defined in the ECSS standard, which is used by ESA and NASA (ECSS-Q-ST-70-57C, August 2013), different combinations of temperature and exposure time have to be used for bioburden reduction depending on the chosen process (humidity controlled or not), the necessary bioburden reduction level, the aim to reduce the bioburden on ambient surfaces or on mated surfaces, or the bioburden encapsulated in spacecraft materials. This has been and continues to be a valuable and practical technique for many types of hardware.

As an alternative, the capability to sterilise the appropriate elements of spacecraft with hydrogen peroxide is being developed (Chung et al. 2008). Recent performance advances in electronics and other thermally sensitive components makes the use of high temperatures unsuitable for some hardware. Experiments to evaluate the effectiveness of vapour hydrogen peroxide (VHP) in a small vacuum chamber system for the inactivation of the standard spore challenge, *Geobacillus stearothermophilus*, were conducted by STERIS Corporation and reported in Chung et al. (2008). Results demonstrated that VHP provided significant reductions in spore viability while allowing survival of sufficient spores for statistically significant enumeration. The study delineated a range of test steriliser process conditions: VHP concentration, process duration, a process temperature range for which the worst case D-value may be imposed, a process humidity range for which the worst case D-value may be imposed, and the dependence on selected spacecraft material substrates. Further work was completed using varying concentrations VHP and chlorine dioxide technologies at ambient pressure in a large chamber (~20m^3) by Pottage et al. (2012). This test was conducted with a range of Bacillus *spp.* isolated from spacecraft assembly clean rooms and studied the inactivation kinetics through the decontamination cycles. The derivation of D-values from the lethality data permitted conservative planetary protection recommendations, which have been incorporated into the ECSS standard ECSS-Q-ST-70-56C (August 2013), which is used by ESA and also by NASA summarizes the experimental details for using VHP for bioburden reduction in both vacuum and non-vacuum chambers.

The sample collection and containment tools, once sterilized, will need to be isolated from other parts of the spacecraft. To isolate the components and systems after sterilization, lightweight biobarrier material needs to be developed that can be applied to different sized objects, and a number of materials are currently in the test phase. A biobarrier could also be produced that would encapsulate and entire spacecraft and would then be used to protect it from the launch vehicle. Another key development identified is a technique for collecting clean samples from beneath the Martian regolith surface, which might possibly be contaminated by migration of microbes from a "dirty" lander or rover. An interesting alternate path involves investigation of techniques to

chemically tag spacecraft contaminants so that these could be recognized in the sample upon return. The techniques for sterilization of spacecraft subsystems being investigated are heat, VHP, plasma, UV irradiation, and gamma radiation (see e.g. Gersham et al. 2004).

6. Planetary Protection techniques and protocols

In addition to extensive decontamination and cleaning of the outbound spacecraft, the return samples potentially containing extra-terrestrial life requires a container that can be remotely closed, sealed and monitored to ensure the external surfaces are ensured to be free of external contamination, safely launched from the planet, monitored en-route, and opened in an appropriate Sample Return Facility (Rummel, NASA white paper).

According to MEPAG E2E-iSAG, 2012, three distinct classes of samples should be returned in order to have a high scientific return; namely rocks, grains such as regolith and sand, and atmospheric samples. Thus the techniques necessary to detect possible presence of life in samples and that are needed to be present inside the Sample Curation Facility should be able to analyse solids, liquids and gasses.

6.1. Analytical techniques

To define the techniques that are essential to detect life signs in the samples returned from Mars, a workshop took place at the Scripps Institution of Oceanography, La Jolla, California in February 2012 organized by NASA and ESA on life detection in samples returned from Mars. The workshop was held the days after the Life-Detection Science Conference, which was organized to discuss, to determine and to assess the latest concepts and methods to search for life on Mars samples (Allwood et al. 2013). Many open questions were discussed during the workshop and the main topics were:

- To review the Planetary Protection Draft Test Protocol as reported by Rummel et al. 2002;
- To identify research and technical developments that are necessary to establish and execute a future Planetary Protection Test Protocol;
- To identify major issues that might affect the requirements necessary to design future sample return flight hardware.

A general consensus was achieved among the members of the Working Group in defining a sequence of analyses to be executed both during samples acquisition on the Martian surface and when the samples are inside the Sample Curation Facility. The suggested analyses were defined to ascertain the presence of life in the samples and the techniques proposed should be able to characterize solid, liquid and gaseous species. The proposed sequence of analyses - as published in the workshop report by Kmineck et al. 2014 - are here showed in Table 1.

Table 1. Sequence of sample analysis to be performed on Mars surface during sample collection and on the samples once delivered to Sample Curation Facility as showed by Kmineck et al. (20014).

Sequence for sample analysis	Sample condition	General type of analysis
I	Sample acquisition on Mars	Remote and *in-situ* analysis on Mars to characterise the sample type and the geological context
II	Any solid sample material on the outside of the sample containers	Solid sample analysis; full sequence (non-destructive & non-invasive, non-destructive & minimal invasive, and destructive)
III	Head space gas	Gas sample analysis; full sequence
IV	Solid samples in containers	Solid sample analysis; non-destructive & non-invasive
V	Solid samples removed from containers	Solid sample analysis; non-destructive & minimal invasive
VI	Fluid inclusions from solid samples removed from containers	Liquid sample analysis; full sequence
VII	Solid sample removed from containers	Solid sample analysis; non-destructive & minimal invasive, destructive

According to the consensus obtained among the participants of the La Jolla workshop, the analytical techniques that were defined as appropriate according to the nature of the samples to detect sign of life on Martian samples were:

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS);

1. Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS);
2. Gas Chromatography Mass Spectrometry (GC-MS);
3. Infrared Spectroscopy (IR);
4. Liquid Chromatography Mass Spectrometry (LC-MS);
5. Scanning Electron Microscopy (SEM);
6. Transmission Electron Microscopy (TEM);
7. Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS);
8. Ultraviolet spectroscopy (UV);

9. X-Ray Absorption Near Edge Spectroscopy (XANES);
10. X-Ray Diffraction (XRD).

As reported in Table 2, the techniques were sorted out according to the nature of the samples. Further considerations were made on the invasiveness of the analyses.

Table 2. Techniques for Life-Detection as defined by the Workshop on life detection in samples from Mars (Kmineck et al. 2014)

Invasiveness	Solid sample analysis	Gas sample analysis	Liquid sample analysis
Non-destructive & non-invasive	• 3D X-ray micro-tomography • Surface imaging and spectroscopy	Not applicable	Not applicable
Non-destructive & minimal invasive (no specific sample preparation)	• Microscopy • Fluorescence • IR, visible, UV, deep UV spectroscopy • SEM	• IR, visible, UV, deep UV spectroscopy	• Microscopy • Fluorescence • IR, visible, UV, deep UV spectroscopy
Destructive (specific sample preparation)	• SEM, TEM, nano-X-ray-tomography • XRD, XANES • GC-MS, GC-IRMS, FTICR-MS, LC-MS, TOF-SIMS, Nano-SIMS • Target independent biopolymer sequencing	• GC-MS, GC-IRMS, FTICR-MS, LC-MS	• GC-MS, GC-IRMS, FTICR-MS, LC-MS, TOF-SIMS, Nano-SIMS, • Target independent biopolymer sequencing, flow cytometry

The detection of any possible life form is based on the paradigm to search of life as we know it. Thus biochemistry that governs all the processes of terrestrial organisms is assumed to be active on Martian organisms. Thus, portion of sample returned will be analysed to detect biosignatures starting from a broad survey of different portions of sample types and continuing with deeper high resolution analysis. To this aim we can organize the techniques showed above according to their capability to provide information on morphological, chemical, biochemical, isotopic and mineralogical signatures. Furthermore, we have here evidenced the specificity of each technique to detect multiple independent signatures. The detection and interpretation of these signatures is the way with which the life detection is based. Thus, techniques and capability to detect biosignatures are showed in Table 3.

Table 3. Techniques for life detection as from Kmineck et al. (2014) are here showed according to their capability to detect biosignatures.

		TECHNIQUE										
BIOSIGNATURE		Electron microscopy - SEM	Electron microscopy - TEM	GC-MS	LC-MS	IR spectroscopy	Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS)	XRD	UV spectroscopy	XANES	TOF-SIMS	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS)
Morphological	Size of single cell - Size of targets	X	X									
	Numbers of single bacteria - Number of targets	X	X									
	Population size (colonies)	X	X									
Chemical	Chemical composition			X	X	X	X			X	X	
	Chirality			X	X		X				X	
	Organic molecules		X			X	X		X			X
Biochemical	DNA, RNA										X	
	Biopolymer (e.g. carotenoids)					X					X	
	Protein										X	
Isotopic analysis	Isotopes, Isotopologues			X		X	X					X
	Isotopomers											X
Mineralogical	Elemental analysis	X										
	Mineral structure							X	X	X		
	Structure, Mineralogy					X		X				

In this project the above list of instruments for life detection is the subject of a review not only by the member of WG 2, that are focused on planetary protection, but also by members of WP4 interested on the characterization of the samples. Implementation is based on the fact that instruments that are identified as essential for Planetary Protection can be also used for preliminary characterization of samples. To this aim we have started to extend the methodologies for life detection defined by Kmineck et al. (2014) in order to encompass the preliminary organic, mineralogy and isotopic characterization necessary to provide the first description of the nature of samples necessary for extended studies that will be performed in worldwide laboratories.

A more articulated approach to life detection and biohazard methods and protocols are presented in NASA/CP-2002-211842 (2002). NASA draft test protocol was developed considering that important effort has to be made to perform a preliminary analysis of the physical-chemical characteristics of the sample to detect signs of life and execute biohazard testing before the samples are released to the broader community for scientific analysis. Isolation and containment of a potential biohazard requires that direct interactions between humans and the sample must be minimized because the most common causes of containment breaches are associated with human handling. The ideal system would provide protection for both the sample and operators of sample curation facility. Largely or totally autonomous sample handling and analysis capabilities are needed, reducing the parallel risks of inadvertent loss of Mars sample containment and sample contamination.

The approach that was used in NASA draft test protocol for detecting life is based on omni-comprehensive methods that not only can identify the organic and geochemical signatures but also is able to evidence silent or still active metabolism or replicating activity. Biosignatures are, thus, identified and can be summarized as in Table 4.

In order to rule out any terrestrial contamination of the sample standard microbiological examination procedures (e.g. cultivation, amplification techniques such as polymerase chain reaction, sequencing methods) should be applied to aliquot of samples. This implies further complexities in designing dedicated environments inside the sample curation facility that must be of high containment, i.e. as those typically used in BSL 4 facilities. The protocols developed to detect life should be reviewed

periodically as part of the ongoing processes that will incorporate new laboratory findings, advances in analytical methods and containment technologies.

Table 4. Biosignatures for life detection (modified from NASA/CP-2002-211842)

BIOSIGNATURE	
Morphological	Size of cell
	Shape of cell
	Structure
	Morphological indication of replicator or specialized features
Structural chemistry	Membrane
	Attachment and motility structures
	Polymer
	Organics or complex molecules
Metabolism and Bioenergetics	
	Products result from specific enzymecatalyzed reactions
Biosyntetic Mechanism	Protein-enzymes
	Protein
	Amino acids
	ribozimes
Isotopic analysis	Isotopes, Isotopologues
	Isotopomers
Geochemical Signatures	Magnetite
	C,H,N,O,P,S in pigments
	Iron, Molybdenum, Tungsten

6.2. Biohazard

Samples returned from Mars have to be considered carrier of agents that can pose threat to life on Earth. Since potential hazards can be of various nature, e.g. chemicals, antibiotic-resistant bacteria, rapidly-mutating viruses etc., the methods and protocols to carry on in the sample curation facility should be diverse. To prevent any threat to personnel operating inside the facility and to environments, development and refinement of high containment areas in which infectious microorganisms could be safely handled is mandatory. The needs to work with tissue cultures, maintain sterility of cell lines, and minimize cross-contamination contribute to concerns regarding

activities plan and facility design. Since the workers are the main source of contaminants (Table 1), samples have to be protected from personnel contamination further than to protect the worker from the risks associated with the manipulation of potentially hazardous materials.

Areas of the facility should be dedicated to whole-organism animal and plant *in vivo* tests and cellular assay and molecular biology *in vitro* tests. Since samples should be considered as dangerous exotic agents that may pose high risk to life-threatening disease, maximum containment equipment (BSL-4) should be used for all procedures and activities. This means to have separate building or isolated zones where biohazard tests will be carried on. Supplies will, thus, be dedicated decontamination system foreseen. However, the ongoing approach is to reduce the use of animals in biohazard testing replacing by cellular and molecular analyses.

The use of proper procedures and equipment cannot be overemphasized in providing primary personnel and environmental protection. The most important piece of containment equipment, however, is the biological isolation cabinet in which robotic and/or human manipulations of hazardous agents are performed. The safety cabinets, within which the maximum level of cleanliness should be maintained, will provide the primary barrier between the sample and the environment. Implementation details discussed in previous studies vary considerably according to number, connectivity, approach to sample handling, and so on (Beaty et al. 2009).

The Draft Test Protocol (Rummel et al 2002) refers to conventional whole organism animal and plant in vivo testing. Thus, a significant part of the floor space of the containment laboratory core should relate to animal holding. Since the Draft Test Protocol was published in 2002 the fields of microbiology and biohazard detection have advanced markedly. To this aim a dedicated workshop is foreseen to be held in Firenze 14th -16th June 2016 addressing the state-of-the-art practices and future implementation. Thus, the design of the sample curation facility would accordingly be simpler.

Three concept studies for infrastructure of a Mars sample receiving facility was investigated and discussed by Beaty et al. (2009) with main aim to define basic attributes. The main activities that

were foreseen in such studies are to receive the Earth Re-entry Capsule (ERC) directly from the landing site with preliminary check and external surface cleaning, disassemble the ERC extract the sample canister from the ERC and initiate subsampling and physical tests, extract the samples from the canister and initiate the life detection testing, perform biohazard tests on samples and finally store samples in pristine environments. The sequence of actions is summarized in Figure 1.

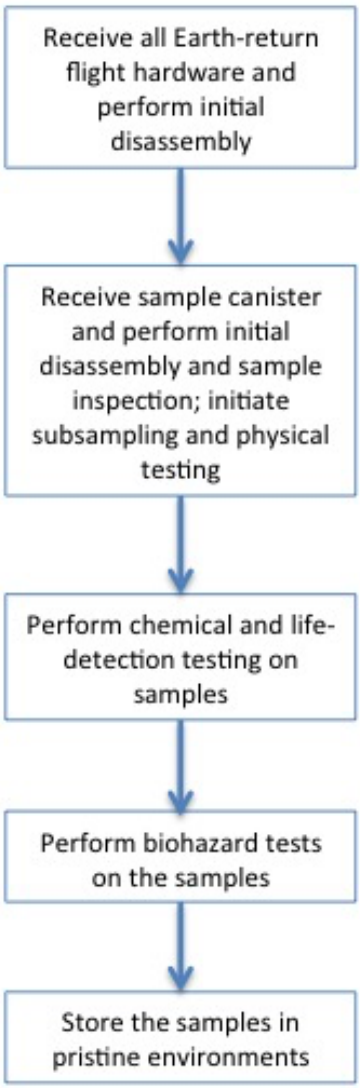

Figure 1. Sequence of actions to be performed in the Sample Curation Facility (modified from Beaty et al. 2009)

The SCF is designed in our view to perform also preliminary characterization analyses of the samples, to catalogue the samples as well as to enwrap the sample containers to send them in to other

facilities and worldwide laboratories. Thus, a further activity we envisage to be added that is missed in Beaty et al (2009). After biohazard tests, sample characterization and cataloguing has to be performed before to prepare the samples for their storage.

These requirements lead to the definition of operational and architectural specifications for a sample curation facility that would be handling potentially contaminated returned samples (Mancinelli 2000, Mani et al. 2007, Allen et al. 2011). This facility requires the combination of engineering technology and interventions used within high containment microbiological laboratories (BSL-4) to maintain the safety of the workers and environment from the sample being handled, with cleanroom engineering and technology to maintain the sterility of the sample from the Earth environment. This type of containment is stringent, including (but not limited to) such requirements as; having the facility under negative pressure in comparison to the external environment (see Figure 2), all personnel change into protective clothing before entering the facility, entry and exit through air locks, all personnel must shower before exiting, all air leaving the facility is filtered to assure no biological agent is released.

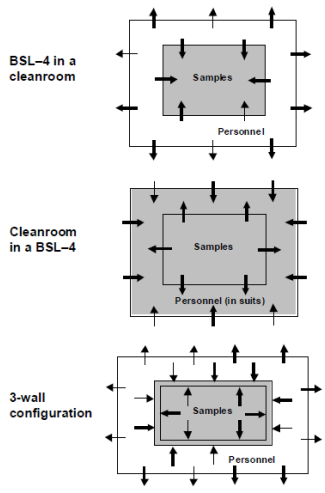

Figure 2. Different options for the combination of a high biological containment facility with a cleanroom design (Rummel et al. 2002).

7. Conclusion

Conclusions from three conceptual studies summarized by Beaty et al. (2009) stated that most of the technology needed for the design and to construct a Mars SCF already exists within the biosafety, pharmaceutical, and sample curation communities. But some technologies and techniques, such as surface decontamination, double-walled glovebox containment and dexterous ultra-clean robotics will need to be developed. Once adequate records of chain of custody of samples are ensured, the tools and instrumentation would be accommodated inside cabinets or on laboratory benches, depending on the specific approach to the containment barriers and sample manipulation. This is subject of investigation in this project. A further specificity that needs to be developed is the interfaces between life-detection instruments and double-walled environments where the samples are contained. Off-the-shelf instrumentations are designed to be operative in common analytical laboratories thus, a further detailed study how to modify analytical instruments to be accommodated within double or single walled chambers is needed.

Materials by which instrumentation is built (e.g. electronics, plastic, metals, pumping system, gas supplies etc.) are not conforming to ultra clean and sterile environments like those envisaged for sample curation facility. Tools in contact with samples, and the sample chambers where life-detection instruments will be placed would need to be cleaned to the same standards as for the sample-contact surfaces. Standards and protocols to achieve this were not developed yet and, thus, it's a matter of further investigation in this project. Instrumentation for life detection will affect the facility design and, thus, they will need to be known before the construction of the facility.

Methodology for biohazard assessing for the detection of pathogenic organism needs to be reviewed and implemented according to new findings that once validated would replace many current practices. Microbiology and biohazard detection have advanced markedly in last years encouraging in vitro tests instead to conventional use whole organism animal and plant in vivo testing. This will largely simplify the design of the facility. Under this project, a dedicated workshop is planned for

accounting on new findings in biohazard assessment (14th -16th June 2016, Florence Italy). The topics to be discussed will be:

- Techniques and methods of risk assessment of biohazard;
- Test methods for the detection of pathogenic organism;
- Containment systems;
- Sterilization processes;
- Safety equipment, enclosed containers;
- Procedures to monitor the health of personal and select precautions for safe practice.

Planned sampling methodology and sample preservation is matter of further study due to the fact that some chemicals decay on exposure to light, or microbes may induce decomposition of organic compounds at ambient temperatures. Moreover, chemically reduced compounds may oxidize on exposure to a head space of air and volatile compounds may escape from an insufficiently sealed vial.

Analytical instruments for life detection as defined by Kmineck et al. (2014) will be subject of review. Many techniques could be used both to detect biosignatures and to characterize samples as mineral contents, petrology, isotopic and organic composition. Thus, a collaborative interaction with WP4 will implement current state of art in facility endowment.

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