



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 640190

**EURO-CARES: A Plan for European Curation of Returned
Extra-terrestrial Samples**

WP6.4: The Impact of Planetary Protection

TN6.4-PHE-01

Written by	Thomas Pottage
Verified by	Allan Bennett
Approved by	Allan Bennett

Approval evidence is kept within the documentation management system.

CHANGE RECORDS



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 640190

ISSUE	DATE	§ CHANGE RECORDS	AUTHOR



TABLE OF CONTENTS

1. INTRODUCTION	5
1.1 Aims and Objectives	5
1.2 Scope.....	5
2. RISK ANALYSIS / ASSESSMENT.....	6
2.1 What is the possibility of life in a sample?.....	6
2.2 Risk mitigation of return missions	6
2.2.1 Capsule design	6
2.2.2 Impact forces with Earth	6
2.2.3 Sample composition.....	7
2.2.4 Exposure to Earth's atmosphere.....	7
2.2.5 Decontamination of the landing site.....	7
2.3 Assessing the risk.....	7
3. BIOCONTAINMENT OF THE EARTH RETURN CAPSULE.....	9
4. LANDING	11
4.1 Nominal	12
4.2 Non-nominal.....	13
4.3 Planetary protection considerations of the landing site.....	13
4.3.1 Backwards contamination	14
4.3.2 Forwards contamination.....	15
5. COLLECTION OF THE CAPSULE AND PRELIMINARY SITE SAMPLING	17
5.1 Meteorological conditions of the site.....	17
5.2 Background sampling of the landing site.....	17
5.2.1 Environmental sampling.....	17
5.2.2 Sample analysis.....	17
5.3 Protective measures around the landing area	18
5.4 Collection of the ERC.....	18
5.5 Portable covering of the landing site	22
5.6 Strategies for decontamination of landing site.....	24
5.7 Decontamination of suits and personnel.....	27



6. STAFF SELECTION, TESTING AND TRAINING	28
6.1 Staff selection	28
6.2 Health Surveillance	29
6.3 Training	29
7. SUMMARY AND RECOMMENDATIONS	31
8. REFERENCES	32



1. INTRODUCTION

1.1 Aims and Objectives

Objectives: To assess the impact of planetary protection and biocontainment on the recovery, initial inspection procedures and the transport to the curation facility. To determine how the procedures for recovery will differ in the cases of restricted and unrestricted Earth return missions.

The document will address specific objectives such as:

- Identifying actions to be taken for nominal and non-nominal returns
- Selection of staff and training requirements
- Discussion of recovery procedures and technology
- Investigations into decontamination options

1.2 Scope

This document will predominantly focus on restricted Earth return missions because these will pose the highest planetary protection impact. While a nominal landing recovery will still need to be carefully planned to ensure compliance with planetary protection requirements this will be limited to protection of the Earth Return Capsule (ERC) from terrestrial contamination. The ERC will need to be protected from gross contamination from the landing site, workers and during transportation. However, this will be at a low impact because of the engineering controls and design of the containment layers within the ERC and the cleaning procedures that will be undertaken within the sample return facility. For category V restricted Earth return missions, planetary protection guidelines state they must have:

“Containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body” and “Containment of any unsterilized sample collected and returned to Earth. [1]”

The major risk will be from a non-nominal landing where there is damage to one or more containment layers of the ERC and potential for release of sample material (potentially containing extraterrestrial life) into the Earth's biosphere and therefore backwards contamination (in addition to forward contamination where Earth lifeforms are transferred to another celestial body or samples from that body). This will not only potentially impact on the pristine nature of the samples, reducing the scientific integrity of the mission and potentially invalidate any further testing during the project, but also potentially release extra-terrestrial material on Earth with unknown consequences. Therefore the majority of this technical note will focus on restricted missions with a non-nominal landing.



2. RISK ANALYSIS / ASSESSMENT

2.1 What is the possibility of life in a sample?

Analysis for the potential of life being present on extraterrestrial bodies has previously been carried out (TN2.1 and TN2.2, Report on protocols, methods and techniques for life and biohazard assessment, and Biohazard and Biosecurity, respectively), and has been used to identify the bodies where no indigenous life is present and also those bodies where life could exist at present or in the past [2]. Any material returned from such a body will be treated as a restricted Earth return mission as it may have the potential to contain extraterrestrial life. If the sample does contain life then it will be unclear what its potential impact could be on any Earth species or environmentally. Pathogenic species on Earth, for example bacteria and viruses, have co-evolved with their host organisms to develop the capability to infect the specific areas of the body [3] and the chances of interaction between terrestrial and extraterrestrial life in a returned sample is likely to be very low. However, there may be the possibility that an extraterrestrial life-form may be able to carry out metabolic activities that could impact on a terrestrial environment if it came into contact with a terrestrial energy source.

2.2 Risk mitigation of return missions

Whilst there is a small risk of a release of viable material from a restricted sample return mission if there is a breach of containment. There are a number of factors that will reduce any risk from this incident.

2.2.1 Capsule design

As described in TN6.2 of EURO-CARES (TAU-100395-WP6.2-TN-0001) the Mars sample return mission design will use an ERC designed for hard landing on Earth. The ERC will not use a parachute for slowing descent instead a descent aero-shell design will be incorporated. This design and the numerous containment layers surrounding the sample tubes will reduce the likelihood that any containment breach will happen and therefore release of sample to the Earth biosphere. It is envisaged that numerous tests will be completed prior to mission launch to demonstrate the ability of the ERC to withstand this hard landing against a number of different surface types it would encounter at the chosen landing site. The biocontainer has a design requirement that it will be able to withstand approximately 50g of acceleration. There may also be a test method engineered into the ERC to show that containment has not been breached in the descent to earth and impact.

2.2.2 Impact forces with Earth

As the envisaged return missions will utilise hard landing of the ERC then this will potentially reduce the viable population of any lifeform in the return samples from the impact of the ERC with Earth. Impacting organisms at high velocities into a solid semi solid surface will cause pressure waves and heat that can inactivate the organisms or components within them (SterLim, Feasibility studies and tests to determine the sterilisation limits for sample return planetary protection measures, in response to ESA call RFQ/3-14132/14/NL/HB).



2.2.3 *Sample composition*

Of the samples returned during the mission the majority will be rock cores and small rocks mixed with regolith. If bacteria are present it can be assumed they will predominantly colonise the external surfaces of rocks and larger particles of regolith. The conditions for the formation of rocks, high temperatures and pressures, will destroy organisms leaving no organisms inside. Bacteria can penetrate rock through pores, cracks and fissures, but this will occur after rock formation and be reliant on the entry point having a larger size than the lifeform to allow ingress. Water can aid the penetration of bacteria into basalt by carrying the cells through pores if the size is adequate to allow passage. For the collection of samples if cored samples are collected then potentially only the surface would contain lifeforms and this would reduce the number of organisms that could be present in the sample if any at all.

2.2.4 *Exposure to Earth's atmosphere*

It is likely that the terrestrial environment may be toxic for any microorganisms existing in an extraterrestrial environment. Organisms that thrive in a high carbon dioxide and low oxygen environment (capnophiles) may find high levels of oxygen toxic like many capnophiles found in environments on earth [4]. Therefore if containment was to break on the ERC and Earth atmosphere was to contact the sample then the level of oxygen could kill any organism present, eliminating any potential risk from backwards contamination. It should be noted though that some organisms are capable of aerobic respiration and fermentation so can survive in either oxygen or carbon dioxide rich atmospheres [5].

2.2.5 *Decontamination of the landing site*

The ERC may be engineered to have sensors within it to detect if there has been a loss of containment and release of sample e.g. pressure sensors. This will allow the retrieval teams to identify early in the recovery phase if measures are needed to decontaminate the landing site (or a wider area). This process will be discussed later in Section 5.6 of the technical note. Decontamination of the landing site will help to reduce the risk from a small scale contamination event limiting further spread of any potential extraterrestrial lifeform.

The considerations discussed above show that the risk from the release of extraterrestrial life from a returned sample, if it is present in it at all, can be reduced further by a number of factors that mean an organism (or population) would be reduced in viability.

2.3 **Assessing the risk**

Planetary protection requirements state that samples from restricted return missions must be handled and treated to the same standards as biological agents within a biosafety level 4 (BSL4) laboratory until it is proven that no extant life is present [6]. Therefore the agent risk assessment has already been undertaken. However, when a restricted return sample curation facility is designed a risk assessment process is required to ensure it is correctly designed to prevent release of any agent.



Structured facility risk assessments are widely used in many industries and have been adopted by high containment facilities. A number of different risk assessment methodologies are available to ensure a high degree of safety assurance. These include Structured what-if technique (SWIFT), Hazard and operability study (HAZOP) and Layers of protection analysis (LOPA) [7, 8]. These risk assessment methodologies are discussed in more detail within TN2.2 Biohazard and Biosecurity. These are used to identify any areas of vulnerability in the design of a containment facility and to identify additional measures that may be required to ensure they will prevent the release of an agent. These often involve engineering additional backups to pre-existing controls.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 640190

3. BIOCONTAINMENT OF THE EARTH RETURN CAPSULE

The ERC design and updates for future missions have been reviewed in the TNs 6.2 'Recovery and Inspection of the Sample', TAU-100395-WP6.2-TN-0001, and 6.3 'Transport to Curation Facility.' These describe the basic design for an ERC and highlight some of the engineering designs that can be used. TN6.2 'Recovery and Inspection of the Sample' describes the basic capsule philosophy for a Mars sample return mission is to 'break the chain of contact' between the Earth and Mars. This is engineered into the ERC by using a number of different containers housing samples that are then sealed within larger containers creating barriers to stop any sample material from being released or conversely any Earth contamination from contacting the samples. The analogy of a Russian doll can be used to describe this approach. This system is also used in the microbiological field to ensure that pathogenic samples are not released from containment; so-called triple packaging [9]. The current concept of the sample container within the ERC is shown in Figure 2, where samples will be collected into individual sample tubes, these will then be placed into the sample cache which in turn is then placed into the orbital sample cache, which is then sealed within the biocontainment system. Finally this will be placed within the ERC for transfer and entry to Earth's atmosphere.

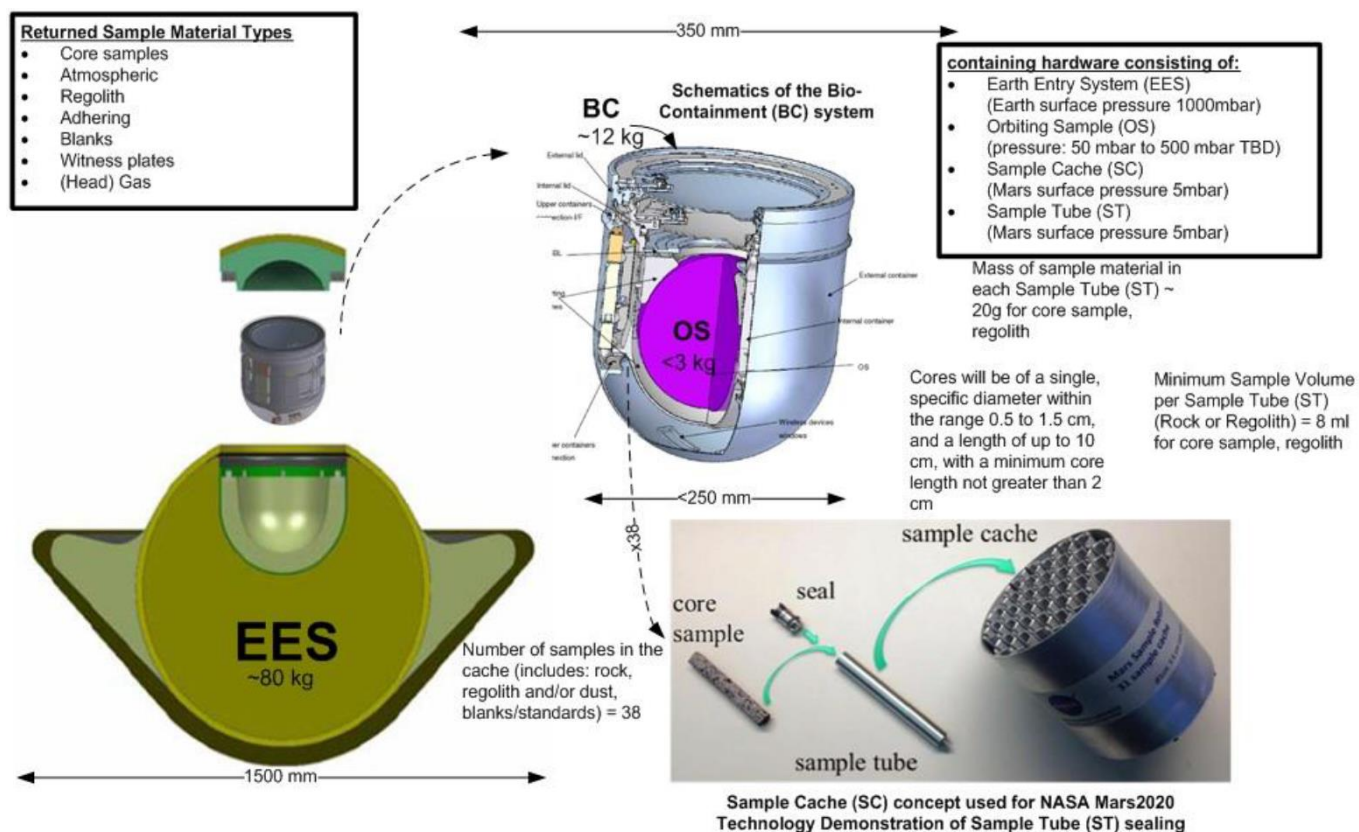


Figure 2. Returned sample material types and container hardware. Image credit TAU-100395-WP6.2-TN-0001



The sample tubes will be exposed to a number of different pressures during the course of the mission. The initial pressure within the tube will represent the atmospheric pressure found on the celestial body, such as Mars. Then when the sample container is in space this external pressure will be reduced in comparison to the sample tube. Then on entry into the Earth's atmosphere the external pressure will be higher thus the sample tube will be at negative pressure to Earth. The current design of sample tubes are currently being pressure tested to investigate the most appropriate tube and seal material [10]. These need to provide a low leak rate for the tubes whilst also having minimal off-gassing that might taint the sample with chemicals. The design of the biocontainer may accommodate a monitoring system to identify any breach in containment. These will measure the pressure within the biocontainer and will transmit the information to mission control and/or the recovery team so containment can be assessed during the landing process. A measurement in the level of pressure change from that which is expected will help determine the size of the breach in containment.



4. LANDING

There are a number of different landing approaches that can be used in returning samples to Earth with the ERC. The ERC can be designed to use an active descent system that will employ engineering approaches to slow its velocity before impact with Earth in the designated landing area. This could be by means of a parachute deployed after entry into the Earth's atmosphere slowing the ERC at velocity that will allow for nominal contact with the ground. This approach has previously been used for a number of return missions, such as Genesis [11], but it does have drawbacks.

Failure of the parachute's integrity or deployment could lead to a ballistic landing at a velocity that will cause failure or destruction of the ERC containment. This was witnessed during the Genesis mission where the drogue parachute failed to deploy after an accelerometer had been installed incorrectly and the ERC was only slowed down by its own air resistance, leading to a ballistic impact which the capsule was not designed to withstand [12], Figure 3. This would present a serious problem in terms of contamination of the immediate area with the returned samples and more widespread contamination from environmental factors (e.g. wind) if particles were small enough to be dispersed (Figure 4).



Figure 3. The genesis capsule after impact with the ground. Image credit NASA

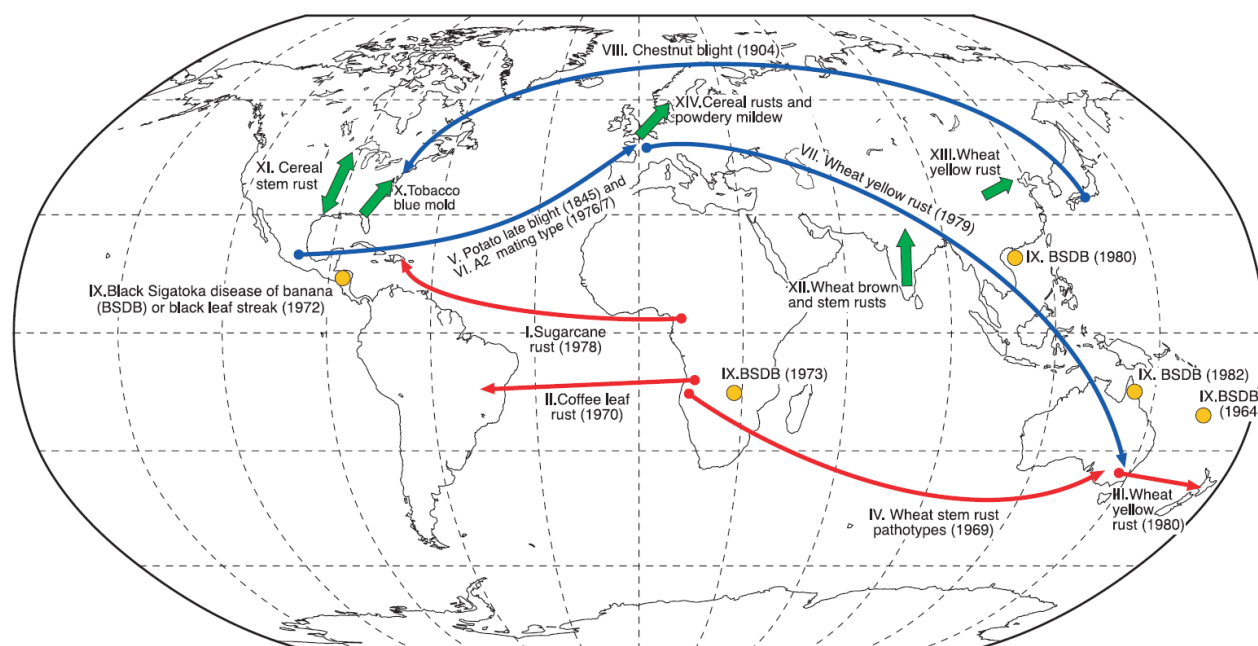


Figure 4. This figure shows examples of dispersal of fungal pathogens around the globe. The red arrows show potential wind dispersal of airborne spores. Green arrows indicate periodic migration of airborne spores (extinction-recolonization cycle and blue arrows show possible spore transportation by infected plant material or humans, and spread further via the airborne route [19].

During the Stardust mission, after successful deceleration with a parachute, the ERC rotated onto its side on contact with Earth and partially obscured the tracking devices which lead to an extended period before the capsule was found and retrieved [12]. If this was the case for time critical samples then the delay in retrieving the capsule could cause degradation in the samples. Furthermore if there was a failure in the containment of the capsule then there would be a potentially greater release of sample onto Earth.

4.1 Nominal

A nominal landing of the ERC would see the descent and landing of the capsule according to the mission design. This would mean that there would be no release of sample material from the capsule through failed containment and therefore no potential for life transmission to the Earth. The operators would then be able to follow normal protocols for the collection of the ERC, its handling and transport to the sample return facility. The procedures for the collection of the ERC are discussed in TN6.3 'Transport to Curation Facility', and the considerations around the landing site are discussed later in this report.



4.2 Non-nominal

A non-nominal landing is where one or more aspects of the landing procedure do not happen according to protocol. This could range from the ERC landing outside of the determined landing zone, coming to rest in the wrong orientation after landing (as was seen with the Stardust mission), or in a worst case damage to the ERC causing a loss of containment and sample release to the Earth's atmosphere. This type of landing will require extra procedures to be put in place to handle any potential release of extra-terrestrial sample into the Earth's biosphere. A non-nominal landing will potentially expose Earth to the collected sample in the ERC and/or the sample to Earth contamination (both biological and chemical) decreasing the scientific merit of the mission.

Current Mars sample return missions are planned to use an ERC able to withstand a hard landing. But even then there will remain a small possibility that the ERC's containment could be breached during the return phase of the mission or by the impact itself.

The detection of a non-nominal landing of the capsule will firstly be by visual checks on landing to assess whether there has been damage to the ERC. Another detection method could be measurement of any change of pressure within the capsule reported through monitoring and transmission of telemetry data to mission control and/or the recovery team. Several pressure monitors could be used on each of the containment 'envelopes' which may allow a rapid identification of the severity of the non-nominal landing. For instance a depressurisation of the external capsule but not the bio-containment capsule would indicate that the samples would still be intact and pristine, allowing the capsule to be potentially collected as normal. It could identify that a more rigorous cleaning methodology may need to be undertaken when returned to the SRF to ensure no Earth contamination remained on the external surface of the bio-container before opening. If the seals for the container housing the sample vessels have been compromised then the recovery team could be able to identify that potential release of material from the ERC had occurred and the specific procedures for this case could be implemented.

4.3 Planetary protection considerations of the landing site

The primary concern for a planetary protection category V restricted mission is the potential for backwards contamination, through the release of unknown lifeforms (pathogenic or non-pathogenic) to the Earth's biosphere. Landing site selection for the return of samples might change depending on the type of mission, whether they are restricted or unrestricted. This could depend on the environmental or human factors within the landing site, for example if the landing was to impact onto unexploded ordnance within a military training area and detonate it, compromising the sample container within it then the mission would be a catastrophic failure, so another landing site without unexploded ordnance might be more suitable. Previous technical notes 6.1, 6.2 and 6.3 have investigated and identified potential landing sites around the world. Each site has been assessed for a number of different factors, such as: nationality, security of the site, tracking of the ERC. These factors are identified to allow access to the landing sites and select for their appropriateness. Environmental considerations will need to be investigated for the selected landing site(s) that will be taken forward for further investigation. Considerations of the landing sites for planetary protection are investigated within this section.



4.3.1 Backwards contamination

Backwards contamination will only occur if there is a breach of the containment and the sample has been released, this might occur through mechanical failure during the landing process (discussed in WP6.1 and 6.2). Other factors such as corrosion of the ERC or freeze-thaw conditions might degrade the capsule to the point where the samples are exposed to the environment but only if they were not recovered immediately. The use of landing sites with areas that the collection team could not access would be highly likely to preclude them from the mission design because of the importance of the return mission and the possibility of the ERC containment failure after an extended period of time exposed to environmental factors on Earth.

The time of the year when the mission would potentially be returning should be considered. Does the landing site go through a range of seasonal variations in climate which could affect the landing? This could be represented by a wet season that would also have the potential for high winds or electrical storms. Wet weather, whilst leading to a more cushioned impact of the ERC with Earth, could lead to increased spread of the samples during a non-nominal landing. Contaminating biological agents could be spread through the water table after being resuspended within surface or rain water. A high water content within the landing site soil could aid the penetration of returned sample through it, if hydrophilic, reducing the possibility of retrieval and decontamination of this returned sample. If the sample was released from containment and entered a water system or lake then this would cause major problems in containing the release as the sample material would be either removed from the area or quickly diluted, in all probability leading to the loss of the sample. In this case it may be necessary for re-assurance to identify the local population centres and start monitoring for unexpected, or a correlation of, illnesses after a non-nominal landing if it is thought that there has been a release of material.

Samples which may be collected from celestial bodies, Mars in particular, will be from areas where water is not thought to be present but might have been in the past. During times of desiccation and other negative environmental pressures, some Earth organisms, such as endospore forming bacteria e.g. *Bacillus* and *Clostridium spp.* [4], will form dormant spores that are highly resistant to environmental pressures (i.e. desiccation) and can persist for centuries. These endospores then become activated and germinate into vegetative cells on contact with water and certain chemicals [13] and then can proliferate within that environment if the correct conditions are present. As described in previous work packages (WP2 – Planetary protection, Task 2.2 – Biohazard and Biosecurity) the likelihood of pathogenic agents being present in a returned extra-terrestrial sample is very low. But the assumption would be that for life to persist in those areas then they would potentially be similar to Earth's extremophilic organisms [14]. These organisms could therefore be potentially able to form dormant bodies that could exist for long periods of time [15, 16]. Whilst a very small possibility, the contact of these potential life forms with the correct conditions for growth and the presence of water on Earth could lead to their proliferation on Earth. This may lead to consider the landing site that had the lowest amount of water content in its soil and the smallest amount of surface water to be selected for reducing the possibility of growth and dispersal of the sample.

High winds over the landing site may affect the landing in more than one way; it will potentially affect the landing process itself by deviating the ERC from its identified path into an unidentified area or



during/after a non-nominal landing the wind can disperse particles away from the landing area and spread any contamination over a wide area [17-19]. The wind direction and velocity over the landing site should be measured as this will allow the modelling of any sample dispersal during descent or landing.

Dispersal of the sample would vary depending on where the breach in containment occurred. If there was a failure from mechanical reasons or through impact with a foreign object, such as meteorite or space debris, before entry into the atmosphere then the sample might not enter the Earth's atmosphere as it could burn up during entry. Bacterial spores have been shown to be reduced in numbers by 90% through exposure to dry heat at 190°C in 1 second [20] and also can survive the low pressures of space [21]. If entry occurred, with containment failing and dispersal occurred in the upper atmosphere the sample material would be spread over a vast area, carried by the upper atmosphere winds. This would create a massive spread of the sample but conversely would dilute any potential lifeforms over this area reducing the risk of agents or toxins contacting host life, especially if multiple agents were required for infection. This dispersal of the sample to the Earth below would be difficult to reliably model, especially with the small amount of sample that might be returned in some missions (Mars Sample Return would return <500g).

A smaller dispersal area, but leading to a higher concentration of sample, would be witnessed if failure of containment on impact of the ERC with Earth. An initial plume of sample might be released through the kinetic forces of the impact which could then be dispersed by the wind. Alternative dispersal of sample might occur with a non-nominal landing, where the containment is damaged to a lesser degree but which still leads to a containment failure and sample would be released only with the action of the wind over the impact site. This dispersal would potentially be more easily modelled but again still spread over a large area which would make it difficult (if not impossible) to collect the entirety of sample or even decontaminate the entire area where the sample had contacted [22].

A non-nominal landing and release of returned sample may not be only pathogenic to humans, but could interact with the flora and fauna of the landing site. Whilst efforts can be made to restrict humans from the site and, to a lesser extent large native animals, smaller animals and insects could will be difficult (or impossible) to control in the impact site. Where, with a failure of containment on landing these animals could indirectly transport released sample to other areas. These smaller animals and insects are likely to be in abundance at the landing site and would therefore be the first Earth lifeforms that would encounter any released Martian material. Therefore observation and monitoring of these by ecologists might give an indication of any potential release of hazardous material.

4.3.2 Forwards contamination

A non-nominal landing would also have the potential to cause a reduction in the scientific value of the mission to the point of rendering it worthless if the entire sample was lost or contaminated. Forwards contamination could occur on Earth during a non-nominal landing. Similarly to contaminating the sample being taken on another celestial body with Earth lifeforms or chemicals on the technology used for its collection, if there was a containment breach in the ERC then there may be ingress of Earth material to the samples. For example there was a failure during the Genesis mission and samples were contaminated with Earth material during landing.



The environmental conditions of the landing site may cause ingress of Earth material through a containment breach. Samples taken and return from bodies that have less atmospheric pressure than Earth will draw Earth's atmosphere or water into them if the sample container is damaged because of the pressure differential. If the ERC lands in a body of water then this pressure differential will increase with the depth of the water, so a reduced retrieval time is imperative to minimise and potential for forward contamination, or removal of landing sites that contain bodies of water.

As described later in this Technical Note environmental sampling of the landing site and specifically the impact site can allow for the comparison and potential identification of lifeforms, biomarkers or chemicals found in the returned sample and the impact environment.



5. COLLECTION OF THE CAPSULE AND PRELIMINARY SITE SAMPLING

5.1 Meteorological conditions of the site

During the selection of the landing site for the ERC it will be essential to take into account the environmental conditions of the landing site. For instance a landing site which has continually high winds might not be suitable because these could affect the descent path of the ERC, the access to the site and retrieval of the ERC by helicopters and if there is a failure in containment a widespread ground release of sample material to the area.. There may be flooding during rainy seasons which may impact on a landing. These can be mitigated if the landing site goes through seasonal variations and the ERC return is scheduled in a period where there is little wind and/or rain.

Monitoring of the wind during the ERC's descent will be important to allow for determination of any potential sample spread from the landing area if there is a breach of containment. This wind direction and strength would allow sample spread through atmospheric dispersal modelling. By determining the loss of mass from the sample tubes then it would be better understood how far and the concentration of sample spread over the dispersed area. Knowing the scale and spread of sample will help in determining the potential affect for the area contaminated. It will allow monitoring to be carried out to determine any changes with time and for surveillance of any populations or animals affected over the area of spread.

5.2 Background sampling of the landing site

5.2.1 Environmental sampling

Samples of the different substrates from around the landing site prior to and after the ERC has landed should be obtained. These samples could be used to determine if and to what extent any contamination has occurred, whether it is forwards, backwards or both and may be used to mitigate in the case of sample contamination by providing an indication of the contaminating material. These samples should represent the different substrates which are found in the landing site i.e. soil, rock, and water. If the landing occurs outside the planned site then samples will need to be collected after impact.

5.2.2 Sample analysis

Environmental samples collected would be held in appropriate storage conditions and facilities i.e. if samples are from the immediate vicinity of a non-nominal restricted return mission, then they will need to be treated as the returned sample itself, and held until the scientific value of the analysis warrants their processing. Environmental samples may be stored and only processed if there is thought to be contamination to the returned samples and the processing of the environmental samples will lead to determination

Current testing procedures and technologies for pathogenic organisms and components of life are detailed in the Work package 2 – Planetary protection, Technical Notes 2.1 and 2.2, Report on protocols, methods and techniques for life and biohazard assessment, and Biohazard and Biosecurity, respectively. These techniques are able to detect either viable microorganisms or cell markers (biosignatures) that indicates the presence of a microorganism. The selection of one or more of these techniques will allow



the scientists to determine if there is or has been the presence of a microorganism in the sample. However it is difficult to specify what technology should be used as new technologies will emerge before the return of a restricted sample mission.

5.3 Protective measures around the landing area

Many of the landing sites identified in WP6.1 (TAU-100395-WP6.1-TN-0001) are on military land and as such there is restricted access to the general public. This will provide an exclusion zone around the ERC when it has landed and therefore help to limit any exposure to extraterrestrial material if there has been a breach in containment during descent or upon landing. The restriction around the sites will also stop any unwarranted attempts to obtain the samples from non-mission staff.

5.4 Collection of the ERC

The collection, packaging and transport of the ERC should be completed as quickly as possible. It has been discussed in TN6.3 'Transport to Curation Facility' that the ERC will remain sealed during these procedures and only opened at the sample receiving facility. This will limit the amount of potential forward contamination as only the exterior of the ERC will have come into contact with Earth and if a nominal landing has occurred then no contamination should be present on the interior surfaces. As discussed earlier in this TN the ERC and sample container will be under negative pressure in comparison to Earth's atmosphere. Sample tubes are currently being developed with current designs achieving a pressure loss of 10^{-7} atm-cc/second of helium, when the internal pressure is held at that equivalent to Mars (approximately 6 mbar) [10]. This shows that pressure will leak into the sample tubes over time and potentially will cause sampled material to be contaminated with Earth material. Although the leak diameter is likely to be small enough to prevent the transfer of any identified lifeforms on Earth.

The collection of the capsules returned from previous sample return missions have all been completed by scientists and engineers. All the previous missions have been unrestricted return where forward contamination from the environment and workers has been the only concern. The retrieval of the ERC after a nominal landing for a restricted return mission would present the same issues as an unrestricted mission. It would be necessary for the collection team to ensure any batteries are disconnected and made safe before packaging in the transport container, no pyrotechnics are envisaged being used on the current design of the ERC. Unlike previous missions, no cleaning or opening of the ERC to remove the sample canister will be undertaken outside of the SRF.

As such the only personal protective equipment (PPE) necessary for the collection team was that to; a) protect against any small explosions whilst making the batteries safe for transport, or b) PPE that will prevent human contamination of the ERC during handling and packaging. The PPE used by JAXA scientists for the collection of Hayabusa fulfilled these criteria and is shown in Figure 5.



Figure 5. Hayabusa return capsule safing of batteries (Image credit: JAXA)

The selection of PPE for staff collecting and ERC after a non-nominal landing will depend on a risk assessment. The selection of PPE will be related to the estimated level of operator protection required, which in turn will depend on the likelihood of release of returned material and potentially against any decontaminant used. For example if one level of containment has been breached in the ERC then this would still not indicate a release of material to the atmosphere, this in turn would mean that normal PPE could be worn for collecting the ERC. If however there was a catastrophic failure of containment on landing then PPE that affords greater protection to the collecting staff should be selected.

Currently the highest level of protection given against contaminants to their wearers, both aerosols and particulate, is from positive pressure suits with self-contained breathing apparatus (SCBA) [23, 24]. Positive pressure suits are so called because compressed breathing air is fed into the suit from a compressed air source. The inflow of filtered air to the suit causes positive pressure within it, which will stop the ingress of aerosols or particulates. These suits were first developed for the nuclear industry and have since been adapted for use in microbiological high containment laboratories [25]. Other suits can be used that filter air from the environment before it is pumped into the suits. All these suits provide a physical barrier to particulate contamination as well as respiratory protection. Within the UK, the Hazardous Area Response Team (HART) use fully encapsulating suits that require SCBA to be worn by the user, an example can be seen in Figure 6, and is manufactured by Respirix (www.respirexinternational.com). These suits are produced as either single use or with a gas tight zip reusable, with a usable life of up to 10 years with inspection and pressure testing to ensure integrity. The military also use disposable suits and unpowered respirators in response to Chemical, Biological,



Radiological and Nucleotide threats. These suits are designed to be rapidly donned and used for an extended period of time. The suit is sealed round the respirator by the hood in the upper half of the suit.



Figure 6. A GTB reusable gas tight suit. Image credit www.respirexinternational.com

There are constraints on using suits both in the laboratory and in 'the field' these are identified below:

Movement – The suits inflate with the ingress of air and therefore can become cumbersome and restrictive of movement. Previous user experience and training using the suits is advisable

Size – The suits can be specially made for individual users e.g. the wellington boots used in the suits are welded to the main body so cannot be changed if different users want different sizes. Therefore suits should be specific to each user

Dexterity – The gloves used on the suits are thick and hard wearing. When working in an environment where contamination is an issue the user may wear a pair of disposable glove prior to entering the suit in case the main gloves become compromised. If samples are being taken then a further set of disposable gloves can be worn over the suit's gloves to allow changing between samples and a reduction in cross contamination. This means that the operator can be wearing 3 sets of gloves and this will reduce their dexterity [26]. The ERC and the transports containers may need to be designed to avoid any need for fine dextrous movements during collection

Noise – With the ingress of air at high velocities these suits can become very noisy for the operator and reduce the amount of communication. Push to talk and in ear radios can be used to reduce the impact of noise



Duration of work – The use of suits can be strenuous and tiring for the operator, especially in hot or sunny conditions, leading to dehydration and exhaustion. This could lead to a maximum amount of time allowed within the suit which could vary with the environmental conditions. Battery life or gas cylinder capacity also needs to be considered

Removal of Suits – Operators should be trained on safe removal of suits to prevent self-contamination during the process.

The collection of the ERC could be undertaken remotely using robots to reduce the potential for the direct exposure of the workers during the collection process. Currently remote operation of robots is used for the identification and disposal of bombs and improvised explosive devices. The CUTLASS programme designed by Northrop Grumman (Figure 7) shows a robotic system currently used by the UK Ministry of Defence.



Figure 7. A CUTLASS robotic system. Image credit Northrop Grumman.

In Figure 7 above the robotic system incorporates a manipulator arm that, could be modified to allow for the making safe of batteries and to lift the ERC into the transport container, then move the transport container to the transport vehicle. This would remove any direct human interaction with the ERC. A robotic system, e.g. drone, could be used to assess the impact site prior to the collection team encroaching. The robotic system could have a number of cameras to record and relay the images to a base of operations where it will allow decisions to be made as to the appropriate PPE required for the collection team prior to their arrival at the site.



Figure 8. A remote controlled drone with camera. Image credit www.yuneec.com

Fine manipulation tasks will be easily achieved using the robotic system, if the correct adaptations were available for the task. It would be envisaged that a lifting device would be required for the larger sample return missions such as Chang'e 5, with a capsule mass of approximately 600kg. The robot system can be modified further to suit the type of terrain that will be found in the landing site, for example tracks could be fitted as opposed to tyres to allow access to finer soil/sandy areas.

Some disadvantages would be if there was interruption of signal to the robotic system during operation and a team was needed to still enter the area to collect the capsule. This would lead to the same results as a mechanical failure on the robotic system. It may also be easier to make decisions whilst on site so having a manned collection team makes problem solving a lot simpler.

5.5 Portable covering of the landing site

Previous recovery missions have not made use of a portable facility that can be placed over the respective ERC and the immediate area. If the landing is nominal and there is no detection of risk that the containment has been compromised and the environmental conditions allow it, then the ERC can be retrieved and packaged into the transport container. If there are adverse weather conditions, rain or high winds, then covering the ERC landing site can aid the packaging into the transport container by protecting the workers and the equipment from damage. If there has been a non-nominal landing and a loss of containment then a mobile facility placed over the impact site will also help to limit any spread of sample material if there has been a breach of containment from the ERC through wind dispersal or precipitation.

Tents and portable facilities are used in a number of other fields to protect their occupants or the material within. Figure 9 shows a tent used in forensic investigations by police forces. Inflatable structures (Figure 10) can be bespoke made to the user requirements and are currently used in a number of situations to protect the occupants and material inside, such as disaster management and military applications (field hospitals). They can be easily and quickly inflated using a generator to power the fan, and have multiple anchor points to affix them to the ground.



Figure 9. A forensic tent used to cover a crime scene. Image credit www.tents4work.co.uk



Figure 10. An inflatable structure used by Medecins Sans Frontieres for a hospital after an earthquake in Haiti. Image credit www.doctorswithoutborders.org

These tents are provided flat packed and can be erected within a short space of time to provide cover over the landing site. The use of the tents in their current sectors where protection of the contents and floor area are critical, show that they are currently at a high technology readiness level (TRL). This would



mean that they would exhibit a technology maturation level of TRL 5/6 for the space industry, with the capacity to increase rapidly to a mission ready TRL.

After use of the tent or other portable facility they can easily be disassembled and either cleaned and repackaged for future use or packaged and then transported for disposal/incineration depending on what is determine as the most appropriate course of action. Continuing to cover the impact site will allow for more considerations to be undertaken regarding the future of the impact site if a breach of containment has occurred.

5.6 Strategies for decontamination of landing site

It will be important to identify the type of containment breach as soon as possible because this will allow the estimation of the size of the area potentially contaminated. It should be remembered that a release of returned sample will contaminate an area that is already heavily populated with microbial life, this existing life will be adapted for growing and persisting in the specific ecosystem so the novel lifeform (if there is one in the returned sample) will most likely be out competed as it would not be adapted for grow in Earth conditions. After assessment of the potential landing sites within the TN's 6.1 and 6.3 it is assumed that the majority of the surface materials will be soil or dust. With this identified then it is possible to investigate methods of isolation or decontamination for the impact site.

After initially identifying that there has been a release of material the area can immediately be treated in one of a number of ways, for example:

1) If the contamination is over a small area and close to the surface then the contaminated soil can be collected into a container, sealed and transported to a prearranged storage facility (potentially at the SRF) where it will be held until examination of the returned sample is completed. The impact site will be sealed and monitored for a period of time to determine if any changes in soil microbiology or chemistry occur

2) If the contamination is thought to be over a wider area, but one that is practical to decontaminate, then a protective measure is placed over the site and the appropriate decontamination process is implemented

3) If the contamination is spread over a large area that cannot be reasonably decontaminated. If applicable the area will be restricted access and monitored for any changes that might be observed over time

As previously discussed in section 5.2, the analysis of samples collected from the impact site will be difficult to interpret due to the large number of terrestrial microorganisms in the soil. This may lead to the site in each of the cases above being closed and sealed from access for a number of years until the returned samples are examined. If no life is deemed to be in the returned samples then the site can be reopened. With the possibility for life in the returned samples that has the ability to replicate in the landing sites low, then a strategy of impact site monitoring might be the most appropriate. It is already seen that the identified landing sites are remote and in the case of the Utah Test and Training Range there are areas that are of limits due to previous tests that have occurred. It would be necessary to close the contaminated area off for a period of time before the technology for decontamination could be



brought to the impact site, which might not be immediately available depending on the region and size of area contaminated.

The removal of the soil around the impact site and/or area thought to be contaminated will remove the source of the contamination and stop any further spread [27]. Depending on the volume of soil to be removed this task can be either completed by hand or by mechanical action. Larger volumes of soil removal will require a greater storage capacity so this needs to be identified prior to the mission returning. There will be practical limits on the volumes of soil that can be removed from a site and this needs to be determined so this option might only be applicable on a small scale. By collecting returned sample contaminated soil from the landing site this would be viewed similarly to having and transporting the returned sample in the ERC so containment of the soil will need to be addressed. Once collected and safely stored then a decision can be reached on whether decontamination is necessary and if so how it can be achieved.

The decontamination of contaminated soil can involve significant investments financially, timewise and to maintain the environment. As described above, soil containment can be a more viable option than soil decontamination. But there are a number of high level approaches could be used for the decontamination of the landing site. These can be separated into physical and irradiation methods. Chemical methods should not be used as the resistance of any extraterrestrial life will be unknown.

Physical decontamination methods will impart physical energy on the soil of the landing site to inactivate the organisms within it. Such methods include incineration of the soil, but generally these approaches will only be able to process soil in small batches. The costs can be high with a ton of soil estimated to cost approximately \$500 dollars to be incinerated [28]. Mobile incinerators can be transported to the site and used to incinerate soil at the landing site, but this will require a large amount of ancillary equipment to complete. A mobile incinerator may not be able to meet the capacity needed and a more permanent incinerator could be constructed at the site or close to it to reduce the transportation needed for the contaminated material. This approach was decided upon during an anthrax outbreak in a Swedish cattle herd, where an incinerator was constructed on site at a cost of approximately \$9 million to incinerate the cattle, feed, bedding and buildings of the farm, around 1350 tonnes of mass [29].



Figure 11. A Portable SRU 855 soil decontamination unit. Image credit www.gencor.com



Within the laboratory/healthcare setting moist heat under pressure (autoclaving) is used to sterilise equipment that is heat stable [30]. This process has been used previously for the decontamination of soil [31-33], but limitations of soil autoclaving show that with incomplete sterilisation, respiration rates within the soil rapidly return to the untreated levels [34]. But for complete inactivation of bacteria and fungi within the soil tested 2 applications were necessary [35]. Larger autoclaves are available for use, Figure 12, but these are installed in a facility and need large amounts of power and steam to operate, such as provided from Bondtech Corporation, USA. These large autoclaves are able to process approximately 1,000Kg/hr but validation will be required to ensure the cycle parameters are efficacious.



Figure 12 shows large medical waste autoclaves. Note the rollers to allow access of large containers. Image credit Bondtech Corporation, www.medicalwasteautoclaves.com

Ionising radiation (gamma radiation) has been used for a number of years for the sterilisation of soil samples in the laboratory. Gamma radiation in a laboratory scale environment is controllable and repeatable, but requires high level exposure for adequate sterilisation. It was found that for adequate soil decontamination approximately 20KGy was required, but for full sterilisation elimination including radiance resistant bacteria 70KGy is required [36]. This higher exposure might be necessary for soil contaminated with extraterrestrial material where exposure on the origin surface might have continual high levels of radiation and therefore radiation resistant lifeforms might have evolved. Cobalt (^{60}Co) is the most common source of gamma radiation. Using ^{60}Co it was seen that 50 to 60 KGy could sterilise soil [35]. Berns *et al.* used spent fuel elements to irradiate 1.5kgs of soil at 4KGy/hr and 1.3KGy/hr with the



exposure periods of 9 hours and 27 hours respectively, achieving complete sterilisation of the soil with both parameters [32].

The use of gamma radiation for soil sterilisation can be effective if applied in the correct manner, but drawbacks are the processing of small volumes for each batch, long exposure times required and the alteration of the chemical composition of the soil exposed [36].

Chemical decontamination of soil can occur again in small batches when it has been collected or in *in situ* at the landing site by the application of a liquid chemical to the soil. This approach can be costly with vast amounts of chemicals needing to be added to obtain a reduction in microorganism numbers. For example the decontamination of Gruinard Island used approximately 2 million litres of 5% formalin over a 4 hectare (40,000m²) area, equating to 50 litres per m². This still required further direct treatments with formalin for small pockets of spores [37]. This shows that whilst possible to reduce the level of microorganisms, the complete inactivation is difficult and would lead to an extensive campaign.

Alternative protective measures have been used for soil that has been unable to be decontaminated using other means. Capping of contaminated land has been used for small areas to contain bacterial contamination and allow the site to be used for development [27, 38]. This might provide a valid alternative to decontamination and also protect the site from environmental factors that could spread any contamination.

Water

The planetary protection considerations of landing in water are discussed in section 4.3 of this report. It was identified that release of any sample material into a body of water would lead to wide scale distribution of that sample making it extremely difficult to collect and decontaminate the water system. In this case ongoing ecological surveys of the water system would be the only possible approach and it would be extremely difficult to definitely detect ecological impacts. Therefore a landing site should be chosen with the smallest amount of water courses or bodies of water within it.

The analysis of the decontamination methods for the landing site undertaken within this section identifies that whilst it is possible to sterilise an area the methods available are costly, time consuming and are not 100% effective. Consideration must also be given to the environmental impact of the process, it may not be possible to use a chemical that could enter the water course and cause chemical contamination. For these reasons careful consideration needs to be given to the most applicable decontamination methodology for the landing site which will take into consideration the amount of contaminated material released, the area contaminated and the contaminated material.

5.7 Decontamination of suits and personnel

If PPE is required to protect staff during the collection of the ERC then there will be a need to decontaminate the suit to make it safe to remove and handle. The suit can be decontaminated directly



using an application of a chemical or use of wipes (using a buddy system). Then the suit would be safely doffed, bagged and incinerated. Training would be required for staff undertaking these procedures

It would not be envisaged that personnel would require separate showering after removal of the suits unless there had been a breach of the suit. Then an emergency shower could be used to wash the debris from the staff member who could then be placed in quarantine (depending on the severity of exposure and the risk from returned sample contact).

6. STAFF SELECTION, TESTING AND TRAINING

6.1 Staff selection

A number of factors can be used to decide on the selection of staff required for the initial inspection, recovery and transport of the samples to the SRF. It would be envisaged that whilst there would be overlap between each of these activities there would also be differences in the makeup of the teams. This would require a decision at high level whether a person or persons would be involved in more than one of the aspects of the collection and transportation elements. Staff would ideally be selected on their ability for working with the technology needed for each stage of the recovery [12]. Staff with specialist knowledge of the construction of the ERC would be required for the initial inspection and potential identification of any variances from the nominal landing process.

Throughout the landing process it would be necessary to have a multifunctional team available for different purposes. This team must be fully trained and competent to fulfil the following functions:

- Recovery (including initial inspection)
- Transportation

And if required:

- Environmental sampling
- Decontamination

It would be prudent to have expert leads in each field within the team. Whilst a number of scenarios would be used in training exercises, it would be unlikely that every scenario would be covered and therefore experience in the field would be required for the staff.

Selection of staff may depend on the chosen PPE required at the landing site. Certain physical attributes might be required and others selected against. If there is a suspected containment breach then it may be deemed necessary for all of the personnel accessing the site to wear high level PPE, such as a positive pressure suit. Conditions in these suits can be hot and physically demanding so staff members might need a medical test before they can be selected for the team.

A key attribute that should be looked for in staff is the ability to work in teams. It will be required to work using a 'buddy' system where one person will undertake a task that will be watched, checked and documented by another. This will be extremely important for tasks that require records being taken and notes being documented. For example during the process of taking environmental samples the 2 person



team will work together with one person taking the sample and the buddy documenting the procedure, by recording the exact location, sample type, conditions and taking photographs. Another example would be for a complex protocol the buddy can assist the operator by providing details of the protocol steps and therefore making it easier for the operator to focus on the necessary steps.

The number of trained individuals would be determined during the mission design process. It would be advisable to have a number of staff members trained in more than one role so they can replace any individuals that maybe not able to complete their task.

6.2 Health Surveillance

Health surveillance is a regimen of checks a worker must undergo to ensure they are suitable for a role and there are no adverse effects from the job they are completing/substances they are handling. Whilst some staff will only undergo basic checks, staff members that are thought to be at a greater risk from a hazard might undergo more rigorous and frequent checks.

Prior to being selected for a team staff will have to have a medical assessment to ensure that they are fit to carry out any tasks required of them. In particular if they have to wear PPE in a hot climate they will have to be assessed whether they can cope with this task. A full medical examination prior to deployment may be carried out to ensure that any changes caused by potential exposure to extraterrestrial material could be detected.

Whilst workers should not come into contact with any extraterrestrial material there is a risk of a non-nominal landing and release of that material. Therefore workers should be part of a surveillance policy where any deviation in their normal health conditions are immediately reported to the medical officer or line manager. A method of health surveillance used would be to continual monitoring of body temperature and other functions using telemetric devices that are being currently developed. It may be appropriate to undertake blood banking with the workers, where samples of blood are taken at various points before and after handling samples, the ERC or after decontamination, these can be compared to identify if there are any changes to the immune response or blood chemistry.

If a change in the worker's health is detected after working on a non-nominal landing then it may be deemed appropriate to quarantine them until an assessment can be carried out to determine the cause. This might involve monitoring multiple health markers (temperature, immune response, blood oxygen levels, etc.).

6.3 Training

Staff recruited for the team roles will ideally have previously worked in a similar environment. This helps to identify staff members that have the appropriate skills and the required aptitude for the tasks. For the ERC collection teams a number of practice recovery missions simulating an variety of scenarios should



be undertaken. This would be started with desk top exercises, progressing to field exercises, then a full recovery mission of a dummy ERC. Training in this way can be used as part of the team selection process, observing their performance before selection of the final teams and improving working protocols. As with working in a team environment one of the key characteristics required is the ability to work effectively in a team when under pressure. Pressures that could be exerted during training are:

- Time, it may be that there is a time limit where the capsule needs to be recovered before, such as to identify if there has been a breach of containment
- Deviation from the mission plan, this could be simulated by a non-nominal landing
- Environmental conditions, recovery exercises could be completed using staged conditions e.g. high winds.

The use of training activities increases the competency of the worker over a number of different scenarios. This will in turn give the worker translational skills which can be applied to even wider scenarios which may happen and have not been able to be trained for. Increasing the competency of the workers will also have the positive effect of decreasing the risks of a recovery mission, reducing the potential spread of contamination from a non-nominal landing and decreasing the potential contamination effects to Earth from sample release.



7. SUMMARY AND RECOMMENDATIONS

This report details the planetary protection considerations of the landing site for restricted return missions and includes aspects of the selection of staff members, their training and health surveillance that might be required for the collection of the ERC. It builds on the previous documents within Work Package 6: Portable Receiving Technologies, identifying the technologies and techniques that can be employed at present for dealing with a non-nominal landing, specifically where the containment of the ERC has failed and sample material has been released to Earth. As such the major points raised and recommendations are summarised below:

The ERC should be designed to survive a hard landing on its return to Earth (WP6.2 DOC), this will require the ERC to be built to withstand considerable impact forces. Consequently the multiple layers of containment will also be built to withstand these forces reducing the likelihood of failure and release of sample material to Earth. It must be remembered though that even though the potential failure of the multiple layers of containment within the ERC is low, any release of returned material could have severe repercussions for life on Earth.

The potential for life within the returned samples being able to survive Earth conditions is very low. The landing site chosen will already be inhabited by a diverse microbial population that is adapted for growth and persistence in that environment. This means that even with a release of sample to Earth the potential for interactions of these lifeforms would be small.

If there was a non-nominal landing of the ERC and release of material to Earth, then different courses of action could be taken. A release of sample into the atmosphere would cause widespread dispersal of the sample and it would be impossible to decontaminate the area of deposition, and therefore all that could be done would be monitoring of this area.

Impact with the ground and release of material would require initially closing that area to access and protection of the site from environmental conditions. This would then provide time for the identification of the projected spread of material and from this, decisions on the practicality of decontamination could be taken. Environmental monitoring of the site could be undertaken or if the area was small enough soil could be removed and incinerated.

It is necessary to establish the sequence of actions that will be taken for each scenario envisaged during the ERC landing, ranging from nominal unrestricted missions through to non-nominal restricted missions. The identification of scenarios that could present themselves will allow the training of the selected staff and recovery processes for each mission. This will then help to ensure any deviance from the mission plan has been catered for and there is a process to deal with it safely and efficiently.



8. REFERENCES

1. ESA, *ESSB-ST-U-001: ESA Planetary Protection Requirements*.
2. COSPAR, *COSPAR Planetary Protection Policy*. 2002. p. 4.
3. Khoruts, A., et al., *Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea*. J Clin Gastroenterol, 2010. **44**(5): p. 354-60.
4. George, W.L., et al., *Selective and differential medium for isolation of Clostridium difficile*. J Clin Microbiol, 1979. **9**(2): p. 214-9.
5. Gregory, E.M. and I. Fridovich, *Oxygen metabolism in Lactobacillus plantarum*. J Bacteriol, 1974. **117**(1): p. 166-9.
6. Foundation, E.S., *Mars Sample Return backward contamination - Strategic advice and requirements*. 2012, Ireg: Strasbourg. p. 62.
7. Dunjo, J., et al., *Hazard and operability (HAZOP) analysis. A literature review*. J Hazard Mater, 2010. **173**(1-3): p. 19-32.
8. Card, A.J., J.R. Ward, and P.J. Clarkson, *Beyond FMEA: the structured what-if technique (SWIFT)*. J Healthc Risk Manag, 2012. **31**(4): p. 23-9.
9. WHO, *Guidance on regulations for the Transport of Infectious Substances 2015-2016*. 2015. p. 38.
10. Younse, P.d.A., T; Backes, P; Trebi-Ollennu, A, *Sample sealing approaches for Mars Sample Return Caching*, in *IEEE Aerospace Conference*. 2012: Big Sky, Montana.
11. NASA, *Genesis Sample Return - Press Kit 2004*.
12. Barrow, K.C., A.; Faris, G.; Hirst, E.; Mainland, N.; McGee, M.; Szalai, C.; Vellinga, J.; Wahl, T.; Williams, K.; Lee, G.; Buxbury, T., *Sample Return Primer and Handbook*, J.P. Laboratory, Editor. 2007. p. 178.
13. Moir, A. and D.A. Smith, *The genetics of bacterial spore germination*. Annu Rev Microbiol, 1990. **44**: p. 531-53.
14. Mancinelli, R.L.K., M., *Martian soil and Uk radiation: microbial viability assessment on spacecraft services*. Planetary and Space Science, 2000. **48**: p. 1093-1097.
15. Horneck, G., H. Bucker, and G. Reitz, *Long-term survival of bacterial spores in space*. Adv Space Res, 1994. **14**(10): p. 41-5.
16. Dragon, D.C. and R.P. Rennie, *The ecology of anthrax spores: tough but not invincible*. Can Vet J, 1995. **36**(5): p. 295-301.
17. Porten, K., et al., *A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany*. BMC Infect Dis, 2006. **6**: p. 147.
18. Gilsdorf A, K.C., Grimm S, Jensen E, Wagner-Wiening C, Alpers K, *Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005*. Epidemiol Infect, 2008. **136**: p. 1084-1087.
19. Brown, J.K. and M.S. Hovmoller, *Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease*. Science, 2002. **297**(5581): p. 537-41.
20. Molin, G., *Inactivation of bacillus spores in dry systems at low and high temperatures*. J Gen Microbiol, 1977. **101**(2): p. 227-31.
21. Vaishampayan, P.A., et al., *Survival of Bacillus pumilus spores for a prolonged period of time in real space conditions*. Astrobiology, 2012. **12**(5): p. 487-97.
22. Gillespie, R.G., et al., *Long-distance dispersal: a framework for hypothesis testing*. Trends Ecol Evol, 2012. **27**(1): p. 47-56.



23. Kumin, D., et al., *How to choose a suite for a BSL4 Laboratory - The approach taken at Spiez laboratory*, in *American Biological Safety Association Conference*. 2011.
24. Steward, J.A. and M.S. Lever, *Evaluation of the operator protection factors offered by positive pressure air suits against airborne microbiological challenge*. *Viruses*, 2012. **4**(8): p. 1202-11.
25. Walker, J., et al., *A review of biological containment suits used in high containment facilities and by emergency responders.*, in *Textiles for hygiene & infection control*, B. Ed McCarthy, Editor. 2011, Woodhead Publishing Limited.: Cambridge.
26. Sawyer, J. and A. Bennett, *Comparing the level of dexterity offered by latex and nitrile SafeSkin gloves*. *Ann Occup Hyg*, 2006. **50**(3): p. 289-96.
27. Pottage, T.G., E. Shieber, C. Wyke, S. Speight, S. Bennett, AM, *UK Recovery Handbook for Biological Incidents 2015*, P.H. England, Editor. 2015.
28. Boulding, J.R., *EPA Environmental Engineering Sourcebook*. 1996: CRC Press.
29. Knutsson, R.B., V. Elvander, M. Olsson Engvall E. Sweden, K. Sternberg-Lewerin, S., *Managing and learning from an anthrax outbreak in a Swedish beef cattle herd*. *Case Studies in Food Safety and Authenticity*, 2012: p. 151-160.
30. Block, S.S., *Disinfection, Sterilization and Preservation*. 5th ed. 2001: Lippincott, Williams and Wilkins.
31. Urbanek, E.B., M.; Doerr, S. H.; Shakesby, R. A., *Influence of Initial Water Content on the Wettability of Autoclaved Soils*. *Soil Science Society of America Journal*, 2010. **74**(6): p. 2086-2088.
32. Berns, A.E.P., H.; Narres, H. D.; Burauel, P.; Vereecken, H.; Tappe, W., *Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy*. *European Journal of Soil Science*, 2008. **59**(3): p. 540-550.
33. Carter, D.O.Y., D.; Tibbett, M., *Autoclaving kills soil microbes yet soil enzymes remain active*. *Pedobiologia*, 2007. **51**(4): p. 295-299.
34. Nowak, A.W., H., *On the efficiency of soil sterilization in autoclave*. *Zentralblatt für Mikrobiologie*, 1987. **142**(7): p. 521-525.
35. Wolf, D.C.D., T. H.; Scott, H. D. and Lavy, T. V. , *Influence of Sterilisation Methods on Selected Soil Microbiological, Physical and Chemical Properties*. *J. Environ. Qual.*, 1989. **18**: p. 39-44.
36. McNamara, N.P.B., H. I. J.; Beresford, N. A.; Parekh, N. R., *Effects of acute gamma irradiation on chemical, physical and biological properties of soils*. *Applied Soil Ecology*, 2003. **24**(2): p. 117-132.
37. Manchee, R.J., et al., *Formaldehyde solution effectively inactivates spores of Bacillus anthracis on the Scottish island of Gruinard*. *Applied and Environmental Microbiology*, 1994. **60**: p. 4167-4171.
38. Turnbull, P.B., J.; Mann, J., *Stubborn contamination with anthrax spores*. *Environmental Health*, 1996. **104**: p. 171-173.

END OF DOCUMENT