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

PLANETARY PROTECTION

SUMMARY REPORT

WP 2 D 2.6

Issue 1 Rev. 1

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1 Introduction

In this deliverable, the results of the activities that were done for the task 2.1 to 2.5 are summarized.
The aim is to summarize the work done in each WP2 deliverable to gather the main information and results obtained during the previous work.
The idea is to synthesize in an easy-to-read text, to include:

- Description of main key points studied;
- Strong recommendations;
- Description of the methods developed, especially if original and innovative;
- Tables and figures, able to give a quick overview of some key points or recommendation.

2 Biohazard Detection

2.1 Summary of D2.1

Samples collected and returned to Earth must be contained and treated as potentially biologically hazardous until they are declared safe by applying recommended protocols, including rigorous physical and chemical characterization, life detection analyses, and biohazard testing.
There is well-documented history of successful biocontainment of pathogenic and infectious organisms in microbiological laboratories. These facilities typically use primary containment devices; negative-pressure gradients and inwards air flow to prevent harmful materials from being released. They are designed to leak in and as a result, are usually both chemically and biologically "dirty", although high containment facilities will filter the air to remove contaminating organisms.
Similarly, there is a record of successful containment for maintaining the integrity of extraterrestrial and planetary samples. These facilities typically use positive-pressure gradients to prevent contaminants from external environment. They are designed to leak out and thus are ineffective for containment of hazardous materials present in extraterrestrial samples.

Planetary protection must work in both directions: the samples have to be preserved to be as pristine as possible and, at the same time, the Earth environment must be protected from potential hazards.
Since it is impossible to foresee the actual risk factor of returned samples, the facilities must have the most stringent containment level presently afforded to the most hazardous biological entities known on Earth. In conclusion, these facilities will require combining of maximum biosafety containment level (BSL-4) with cleanroom technologies.
The primary objective for planetary protection is to asses Life Detection (LD) and Bio Hazard (BH):

- LD analyses should be based on broad definition for life and an approach for detecting life not limited by the specific features of life, as we know on Earth. This approach will rely on signatures of various types that encompass all known terrestrial life, and that might encompass non-terrestrial life.
- BH testing has the aim of determining if samples pose any threat to terrestrial organisms or ecosystems, regardless of whether the samples contain life forms or non-replicative hazards. Since potential hazards could take a multitude of forms, the spectrum of tests has to be diverse. In practical terms, biohazard testing should determine of whether the samples contain any biohazard and whether to distribute sub-samples while providing a reasonable assurance that the samples will not put humans or other terrestrial organisms at risk.

Given the extreme difficulty of describing all possible living processes, a single approach is not able to guarantee success with a given sample. Multiple approaches are key to the successful detection of possible life in a sample.

2.2 Key Points

D2.1 Main key points			
N.	**Key Point**	**Description**	**Notes**
K1	**Life and biosignatures**	Starting from what is available at the state-of-art, some definition of life and biosignatures have been proposed and categorized: morphology, structural chemistry, metabolism and bioenergetics, biosynthetic mechanisms, isotopic signatures, geochemical signatures-	
K2	**Biohazard**	A short biohazard definition has been provided. Based on the state-of-art, a chemical/biological risk assessment and a classification of risks has been reported.	
K3	**Procedures for LD and BH**	A procedure for LD and BH assessment has been proposed.	
K4	**Techniques and Technologies of analyses for LD and BH**	An advanced approach to correlate the biosignatures with the techniques has been defined. It leads to a hierarchical approach able to define which are the best techniques to be used inside the curation.	
K5	**Sample selection**	To correctly select the samples from the canister a procedure and some amount considerations have been described.	
K5	**Environmental monitoring and security issues**	An overview of the major potential hazard has been performed and summarized into main categories: physical, chemical, containment, environment, personnel.	

2.3 Recommendations

D2.1 Main recommendations			
N.	**Recommendation**	**Description**	**Notes**
R1	**Curation design approach**	Since it is impossible to foresee the actual risk factor of returned samples, the facilities need to have the most stringent containment level presently afforded to the most hazardous biological entities known on Earth. These facilities will require combining of maximum biosafety containment level (BSL-4) with cleanroom technologies	
R2	**LD and BH procedures**	The upper-level procedure should require a sequence of operations.	**Figure 2.1**
R3	**LD and BH outcomes**	Once sufficient information is available for characterizing and understanding the biological materials in question, informed decisions can be taken to: • Downgrade or eliminate containment requirements, if deemed appropriate. • Continue containment of all unsterilized samples for an indefinite period. This applies to two different cases: until a clear evidence of life (or its absence) is defined or until the living organisms are better understood. • Sterilize the samples (sterilization activities are optional and subject to further scientific, technological or even political in-depth decisions).	
R4	**Biohazard analyses**	In case of the detection of a living, self-replicating organism within a sample, biohazard analysis should follow a clearly defined chemical and biological risk assessment: • Hazard assessment: evaluation of the intrinsic hazard characteristics of biochemicals and macromolecules. • Dose-response evaluation: in the case of a biological agent, involves parameters such as minimal dose for infectivity, pathogenicity, environmental transmission, and distribution in the ecosystem populations. • Exposure assessment: such as those involved in occupational, clinical, and general environment-related activities using a set of realistic exposure	

		scenarios. • Risk characterization: a formalized approach to combine the characteristics of hazard, toxicity, and exposure to derive a measure of risk associated with the biological agent.	
R5	**Sample selection procedure**	A hierarchical approach should be followed: 1. Samples exteriors test for organic compounds and any released gases 2. Samples non-destructive methods of analysis to map the microscale spatial distributions of minerals and biological elements in samples; 3. Acquisition of aliquots of samples from most promising areas, targeted by compositional and microtextural mapping, performed at step 2.	
R6	**Amount of samples for LD/BH assessment**	According to the state-of-art, a minimal amount of 1.5 g should be used for LD and BH assessment. If the overall available quantity of samples is higher, this value should not exceed 10%.	
R7	**Techniques for LD and BH**	An objective approach able to make a comparison between the techniques in terms of effectiveness should be applied inside the curation, in order to: • Determine a ranking able to prioritize techniques. • Define which technique is important and what can be considered as optional. • Rationalize the entire activities flow inside the curation. • Provide support for the evaluation of the design choices of the curation.	**Method 2.4.1**

2.4 Methods

2.4.1 Biosignatures vs. Techniques correlation matrix

Many techniques and instruments are able to provide information on the presence of living organisms on returned samples. A quantitative method is desirable to assess the most appropriate set of instruments for life detection. Thus, a correlation matrix was used to correlate the biosignatures with the techniques. It is known that a number of techniques are suitable to detect various biosignatures. Using the correlation matrix, it is possible to summarize all this information at a glance. Once a correlation between a biosignature and a technique is defined, it is also possible to determine the strength of this correlation, giving a value. Here, the scale used for the correlation value was:

- **9** - Very specific technique for the biosignature, with high resolution;
- **3** - Technique suitable for the biosignature, although perhaps not specific, and/or with medium resolution;
- **1** - No specific technique for the biosignature but still usable and/or with medium/low resolution

Another input in the correlation matrix has been chosen to quantify how substantial is each biosignature. This is the "Importance value", rating in a scale from 1 to 4 (see the following table).
The results are shown in the following table, where:

- The biosignatures are organized per area: morphological, chemical, biochemical, isotopic analysis, mineralogical;
- The importance is given for each technique;
- A correlation value is given, if the correlation exists;

The matrix is organized in order to be diagonal, where, for each biosignature, the higher correlation value techniques (9, in green) are written in the first available columns

			TECHNIQUES																											
BIOSIGNATURES		IMPORTANCE	Optical microscopy	Electron microscopy - SEM	GC-MS	LC-MS	MALDI-TOF	Fluorescence microscopy	Raman spectroscopy	High Performance Liquid Chromatography (HPLC)	Polymerase Chain Reaction (PCR)	Enzyme-linked immunosorbent assays (ELISA)	Fluorescenct in-situ hybradization (FISH)	Sequnecing	Chromatography	Protein microarray / Marker Chip	SIMS	Isotope Ratio Mass Spectrometry (IRMS)	13C-NMR	SEM-EDX	XRF	X-Ray CT	XRD	Electron microscopy - TEM	NMR	FTIR	Marker chip with antibody	Capillary Electrophoresis (CE)	MC-ICP-MS
Morphological	Size of single cell - Size of targets	4	9	9				3																3					
	Numbers of single bacteria - Number of targets	4	9	9				3																3					
	Population size (colonies)	2	9	9																				3					
Chemical	Chemical composition	4			9	9	9																		1	3			
	Chirality	4			9	9	3								1										1				
	Organic molecules	4						9	9	9							1			1	1			3		3	3		
Biochemical	DNA, RNA	4									9	9	9	9															
	Organic pigments	4					3		9	3					9											1		3	
	Protein	4					9					9		9		9													
Isotopic analysis	Isotopes, Isotopologues	3			3												9	9								1			3
	Isotopomers	2																	9										
Mineralogical	Elemental analysis	4																		9	3								
	Structure, Mineralogy	4	3					1	3											1	9	9	9			3			

It is important to recognize that some of the techniques able to satisfy the requirements of planetary protection are also necessary to the preliminary characterization of the samples.

The important aspect that we would like to underline is that by using the correlation matrix method we move towards a quantitative evaluation approach in selecting the suitable techniques for LD, able to deal with numbers and indicators.

Analysing the correlation matrix it is now possible to make some observations:

- The initial list includes 27 different techniques;
- The number of high-correlation techniques (given value 9, in green) are 21;
- There are 8 techniques able to detect 2 or more biosignatures;
- If only high-correlation techniques are considered, the minimum number of techniques needed to solve all the biosignatures is 9. This number decreases to 7, if also the medium correlation (given value 3) techniques are considered;
- If only the high-correlation techniques are considered, the minimum number of techniques needed to solve all the high-importance (given value 4) biosignatures is 7. This number decreases to 6, if also the medium correlation (given value 3) techniques are considered.

At this stage of the analysis, the result can help to define the major drivers:

- The matrix allows us to select the most important techniques;
- This leads us to define the procedures to be performed inside the curation, that are strictly related to the techniques;
- Starting from the obtained results it is possible to facilitate the design choices: choosing a technique allows a better evaluation of curation dimensions (depending on the size and

position of the instrument, etc.) and layout (depending on its position, the compatibility with other instruments, the need of auxiliary gear, etc.).

A further analysis can be done where four more indicators are included:

- Biosignature occurrence: shows the number of times that the each biosignature is detected by a different techniques (e.g. organic molecules can be detected by 9 different techniques);
- Techniques occurrences: shows the number of biosignatures that can be detected by a single techniques (e.g. FTIR can detect 5 different biosignatures);
- Non Destructive/Destructive Coefficient: it gives an added value to disentangle destructive and non-destructive techniques, (1 if the technique is destructive, 1.1 if partially destructive, 1.2 if partially destructive/non-destructive , 1.3 if not destructive);
- Technique mean value is the technique mean correlation with the detected biosignatures (e.g. FTIR, that has 5 occurrences with biosignatures, has a low technique mean value equal to 2.2);
- Techniques importance rating is calculated for each column (technique), as the sum of the product of the biosignature's importance, the correlation value and the non-destructive/destructive coefficient (e.g. the optical microscopy technique has the higher value).

Among the previous indicators, the most important is the technique's importance rating, which at the same time is dependent on the biosignature's importance, the correlation value, and the technique's occurrences.

BIOSIGNATURES		IMPORTANCE	Optical microscopy	Electron microscopy - SEM	GC-MS	LC-MS	MALDI-TOF	Fluorescence microscopy	Raman spectroscopy	High Performance Liquid Chromatography (HPLC)	Polymerase Chain Reaction (PCR)	Enzyme-linked immunosorbent assays (ELISA)	Fluorescenct in-situ hybradization (FISH)	Sequnecing	Chromatography	Protein microarray / Marker Chip	SIMS	Isotope Ratio Mass Spectrometry (IRMS)	13C-NMR	SEM-EDX	XRF	X-Ray CT	XRD	Electron microscopy - TEM	NMR	FTIR	Marker chip with antibody	Capillary Electrophoresis (CE)	MC-ICP-MS	BIOSIGNATURES OCCURRENCES
	TECHNIQUES																													
Morphological	Size of single cell - Size of targets	4	9	9				3																3						4
	Numbers of single bacteria - Number of targets	4	9	9				3																3						4
	Population size (colonies)	2	9	9																				3						3
Chemical	Chemical composition	4			9	9	9																		1	3				5
	Chirality	4			9	9	3								1										1					5
	Organic molecules	4						9	9	9							1			1	1			3		3	3			9
Biochemical	DNA, RNA	4									9	9	9	9																4
	Organic pigments	4					3		9	3					9											1		3		6
	Protein	4					9					9		9		9														4
Isotopic analysis	Isotopes, Isotopologues	3			3												9	9								1			3	5
	Isotopomers	2																	9											1
Mineralogical	Elemental analysis	4																		9	3									2
	Structure, Mineralogy	4	3					1	3											1	9	9	9			3				8

TECHNIQUES OCCURRENCES	4	3	3	2	4	4	3	2	1	2	1	2	2	1	2	1	1	3	3	1	1	4	2	5	1	1	1			
TECHNIQUES MEAN VALUE	7,5	9,0	7,0	9,0	6,0	4,0	7,0	6,0	9,0	9,0	9,0	9,0	5,0	9,0	5,0	9,0	9,0	3,7	4,3	9,0	9,0	3,0	1,0	2,2	3,0	3,0	3,0			
TECHNIQUES IMPORTANCE RATING	102	90	81	72	96	64	84	48	36	72	36	72	40	36	31	27	18	44	52	36	36	42	8	43	12	12	9			
NON DESTRUCTIVE/DESTRUCTIVE COEFFICIENT	1,3	1,1	1	1	1	1,3	1,2	1	1	1	1	1	1	1	1	1	1,3	1,1	1	1,3	1,3	1,3	1,3	1,3	1	1	1			
TECHNIQUES IMPORTANCE RATING (*ND/D)	133	99	81	72	96	83	101	48	36	72	36	72	40	36	31	27	23	48	52	47	47	55	10	56	12	12	9			

2.5 Tables and Figures

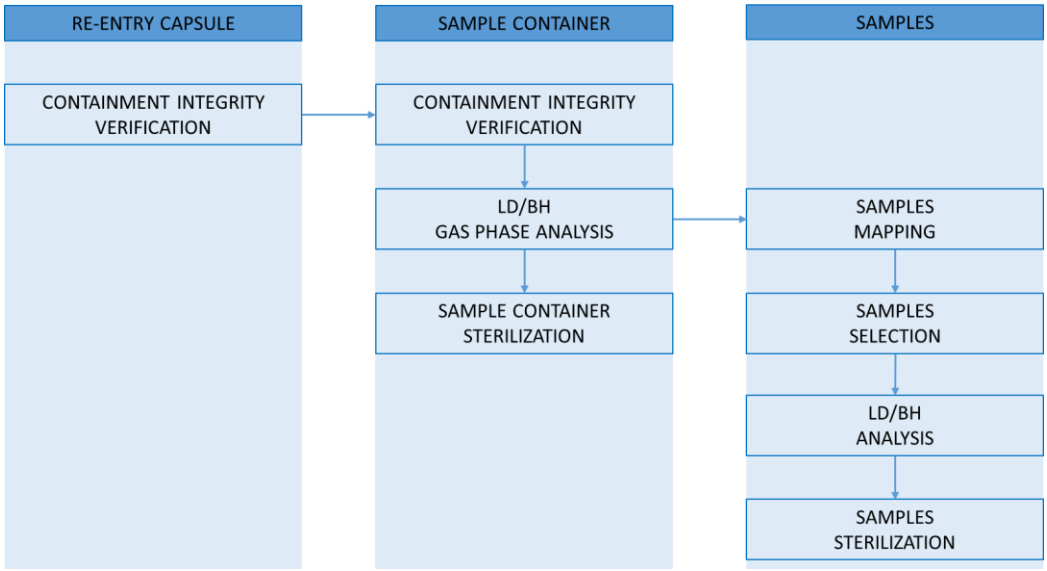


Figure 2.1 - *LD/BH procedures: sequence of procedure used for LD/BH assessment, applied to re-entry capsule, sample container and samples*

3 Biohazard and Biosecurity

3.1 Summary of D2.2

The return of extra-terrestrial samples to Earth will be regulated under planetary protection guidelines. More specifically the handling and investigation of returned samples will need to satisfy category V of the planetary guidelines. Briefly, category V is separated into unrestricted and restricted sections:

- Unrestricted missions bring backsamples from locations judged by scientific opinion to have no indigenous lifeforms, so there are no special requirements to protect the Earth's biosphere, for example samples returned from the moon.
- Restricted missions bring back samples from solar system bodies that potentially contain life, for example Mars. Unsterilized samples and any hardware that directly contacted the target body of samples from that body must remain in containment until sterilized or no life is found in the samples.

The facility that will receive any restricted missions must be able to therefore hold the samples within containment that will stop any release of an unsterilized particle. Specifically the Planetary Protection requirements states that the probability of a single unsterilized particle of ≥0.2 µm being released from this facility shall be ≤ 1×10^{-6}. The highest level of containment currently used for biological materials is the Biosafety Level 4 (BSL4) laboratory. These are designed to allow handling and safe storage of the world's most pathogenic agents. The laboratories are designed to meet requirements defined by international and national bodies, ensuring biocontainment throughout a number of measures, such as, but not limited to, primary and secondary containment, staff selection and safe working practices. The measures already employed for high containment facilities demonstrate that the majority of the technology for a restricted earth return mission is already present, these can be built upon with technologies adapted from the pharmaceutical industry and developing technologies such as the double walled isolator and robotic manipulation.
This section of the deliverable will touch upon the requirements for other work packages that relate to this topic but will focus on the biohazard and security aspects of a facility relating to planetary protection for restricted Earth return samples. As unrestricted Earth return missions will not require containment to protect the Earth's biosphere then they will not be considered within this report, apart from the use of containment systems to protect them from Earth contamination. This section will focus on restricted Earth return missions where the sample will need to be contained from the Earth biosphere to avoid contamination but also to ensure the Earth's biosphere is protected from the sample.

3.2 Key Points

D2.2 Main key points			
N.	**Key Point**	**Description**	**Notes**
K1	**Identification of hazardous agent**	This section identifies that as a rule pathogens on Earth have co-evolved with their host species. So whilst extraterrestrial life forms might be present in returned samples, if they are able to survive the extreme conditions found on those celestial bodies they will be highly unlikely to infect any Earth lifeforms as they would have evolved in isolation to Earth life.	
K2	**Assessment of risk**	A number of different factors need to be considered when performing a biological risk assessment for extraterrestrial samples creation and analysis. Currently there is no knowledge on the agents that might be present so it is very difficult to accurately complete an assessment. As part of this process, it will be necessary to use information from agents present in extreme environments on Earth and on our understanding on how 'life' is defined.	
K3	**Documentation and regulations on containment**	There are currently no regulations detailing the necessary requirements for the construction and validation of a facility to handle restricted return samples. The only regulations that need to be adhered to are from ESA's planetary protection requirements relating to the probability of unsterilized particles ≥0.2 µm being released from the facility, being less than ≤1 x 10^{-6} (ESSB-ST-U-001).	
K4	**Principles of high containment**	This section identifies the key containment engineering and principles that are available for use to handle restricted return samples. This section addresses both primary and secondary containment that is used in BSL4 facilities presently.	
K5	**Considerations for the safe working of a high containment**	Ancillary parameters for the safe handling of hazardous agents such as the decontamination of laboratory waste planned preventative maintenance, showering of staff and PPE, standard operating procedures and biosecurity considerations. These have been explained in the context of a sample return mission	
K6	**Current test methods of life detection**	An explanation of microbiological tests, culture and molecular, that can be completed to determine if there is the presence of life in a returned sample.	

K7	**Health surveillance and staff selection**	There are a range of health surveillance measures that can be currently used to determine the suitability of a worker to work at high containment (eye sight, blood tests, hearing, etc.) and whether the workers might have come into contact with the agent they are working with and become infected (temperature monitoring and blood banking). Staff should be identified by having demonstrated competence at a similar level of containment or having demonstrated the necessary skills which can be transferred to the sample return facility. Further training can then be given to raise the staff member to the competence level required.	

3.3 Recommendations

D2.2 Main recommendations			
N.	**Recommendation**	**Description**	**Notes**
R1	**Identification of hazardous agent**	It will be necessary to identify new microorganisms as they become known, especially if they are found in environments that could represent those found on a celestial body where samples might be returned from. Testing could be undertaken to establish if they are hazardous to other lifeforms that are unconnected to them. Protocols that will be required for methods of identification of hazardous agents need to be updated.	
R2	**Assessment of risk**	As newer microorganisms are discovered and characterised any microorganisms discovered (especially extremophiles) could be used to provide a more specific risk assessment. Novel microorganisms should be examined for their applicability to be used in a risk assessment, for example such as if they are small compared to those already identified.	
R3	**Principles of high containment**	Primary and secondary containment measures have been used to successfully contain high-risk pathogens. At present, the containment measures can be designed, built and validated to accommodate a wide range of tests and equipment. Primary containment (class II and III cabinets) have been designed to confer microbiological sterility to the samples being processed. Containment measures will need to be designed in conjunction with the facility as a whole and with what tests and equipment will be required within it. This will	

		need to be put on the roadmap for any Mars sample return curation facility	
R4	**Considerations for the safe working of a high containment**	It is necessary to identify and validate decontamination approaches that can be used in the laboratory to ensure that every particle that leaves the facility is sterilized. This should be completed prior to the facility being designed because the decontamination approach will need to be incorporated into the containment laboratories. Standard operating procedures should be developed for each procedure to be completed in the facility. Staff members can then be trained on these during the facility and experiment validation phase.	
R5	**Health surveillance and staff selection**	Current health surveillance techniques that are used in high containment laboratories should be followed. These may differ depending on the type of laboratory chosen i.e. either suited or cabinet line. Staff selection will depend on the techniques that will be performed within the facility. It would be envisaged that there will be a period after design of the facility, during construction and then in the validation of the techniques and facility where criteria for staff members will be identified allowing for selection of staff. Staff could either be chosen on those that have experience in the techniques to be performed or with experience in the containment used. Training would then be given to ensure the staff members could demonstrate competence in both fields before any work on received samples is undertaken.	

3.4 Methods

Not applicable

3.5 Tables and Figures

Not applicable

4 Sterilization and Techniques

4.1 Summary of D2.3

The aim of this work package was to review of existing sterilization and cleaning methods and techniques used for terrestrial microorganisms. Furthermore, we aimed to define methods and protocols for sterilizing samples and for cleaning laboratory materials from gaseous, liquid, solid and molecular contaminants. The requirements for sterilization of Mars samples using terrestrial extremophiles as model systems as well as recommendations for sterilization methods and implementation were analyzed and discussed.

4.2 Key Points

D2.3 Main key points			
N.	**Key Point**	**Description**	**Notes**
K1	**Decontamination methods**	Different state-of-the-art decontamination methods were evaluated and described according to their possible applications during the proposed endeavor	
K2	**Cleanroom cleaning technologies**	Standard clean room cleaning technologies were revised and summarized to provide an overview of necessary steps before any sample may be analyzed in the proposed facility	
K3	**Bioburden reduction of space hardware**	Current procedures for the bioburden reduction of space hardware have been revised and compiled to an comprehensive overview	

4.3 Recommendations

D2.3 Main recommendations			
N.	**Recommendation**	**Description**	**Notes**
R1	**Gaseous Decontamination**	The restricted sample return area should be sterilized by the VHP method, to ensure a sterile environment for sample handling	**Method 4.4.1**
R2	**Non-thermal plasma**	This form of decontamination / sterilization needs to be further investigated for its potential use in this endeavor	
R3	**Disinfectants**	The use of disinfectants should follow the guideline EN16615 for all of the facility	

4.4 Methods

4.4.1 Technology trade off selection

A trade off matrix has been constructed to allow for the comparison of the current gaseous decontamination technologies that are considered applicable for use in the sample receiving facility. The parameters they are scored against have been detailed below and then these have been weighted depending on their importance within the sample receiving facility.

Parameter	Weighting	Details of weighting
Biological efficacy	**3**	It is important for the technology to have demonstrated efficacy against a range of microorganisms, especially those deemed as more resistant to decontamination.
Material compatibility	**3**	The technologies should not damage any surfaces or components within the enclosures.
Reproducibility/process control	**3**	It will be a requirement of the decontamination process to be reproducible so each cycle will be the same (within predefined acceptable tolerance levels). The technology's ability to regulate the environmental parameters will make the cycle more reproducible.
Residue formation (including oxidation)	**2**	Residue/surface oxidation formation could lead to false results from tests after decontamination. Residues might affect the performance of equipment.
Cycle duration	**2**	Cycle duration might be important if there is a requirement to decontaminate surfaces or equipment between analysis of samples to avoid cross contamination.
Volume decontaminated by one unit	**1**	The capacity of the technology to decontaminate large volumes at one time can reduce the number of units required. It may be necessary to decontaminate the entire laboratory in an emergency.
Cost (€) per unit (including consumables)	**1**	Equipment for decontamination can be expensive, but the will be a small fraction of the overall facility cost.

The scoring criteria matrix is shown in the following table, where, per each parameter, there is a score, depending on the parameter range/outcome.

	Score		
Parameter	**3 - Excellent**	**2 – Satisfactory**	**1 - Poor**
Residue formation (including oxidation)	None, low oxidation of surfaces	Limited non-organic residues	Large number of residues
Material compatibility	Shown for all relevant materials using accepted standards	Incompatible with limited number of components	Little evidence/ incompatible with large range of relevant material
Biological efficacy	Peer reviewed scientific publications	Limited peer reviewed scientific publication/ independent test reports	Company Funded test reports / advertising Claims
Reproducibility/process control	All environmental parameters controlled & recorded	Some environmental parameters measured & controlled	No environmental parameters controlled or recording process

Cycle duration	<2 hours	2-6 hours	>6 hours
Volume decontaminated by one unit	>100 m^3	–20-100 m^3	<20 m^3
Cost (€) per unit (including consumables)	<10K	11 - 75K	>75K

Finally, the technology trade off matrix is shown, where, for each technology, the value is given by the sum of the score x weighting products. As an example, for VHD, considering the sum of the biological efficacy (given by the product of a score 3 and a weighting 3) the material compatibility (given by the product of a score 2 and a weighting 3), etc., the final value is 40.

		Technology					
Parameter	**Weighting**	**VHP**	**HPV**	**EO**	**CDG**	**Formaldehyde**	**Aerosolized hydrogen peroxide**
Biological efficacy	3	9	9	9	6	9	6
Material compatibility	3	6	6	6	3	6	3
Reproducibility/ process control	3	9	6	9	9	3	3
Residue formation (including oxidation products)	2	6	6	2	4	2	6
Cycle duration	2	6	6	2	4	2	4
Cost	1	2	2	2	1	3	2
Volume decontaminated	1	2	2	1	3	2	2
TOTAL	**(46)**	**40**	**37**	**31**	**30**	**27**	**26**

The technology trade off shows that all the scores range from 40 to 26 points. Steris's VHP technology scored the most points in the trade off with 40 out of 46, followed by Bioquell's HPV (37), ethylene oxide scored 31, chlorine dioxide 30 points, followed by formaldehyde with 27 points and aerosolized hydrogen peroxide scored the least with 26 points.

4.5 Tables and Figures

Not applicable

5 Sample Transfer

5.1 Summary of D2.4

Once recovered from the sample collecting mechanism, returned samples should be moved from the canister to the working area where they are analyzed, stored and delivered to external laboratories. In general, there are three possible transfer paths:

- Inside the ESCF;
- From the ESCF to external locations (i.e., external laboratories, other curation facilities, etc.);
- From external locations to the ESCF.

Some specific technological solutions should be defined for each path, able to satisfy both requirements:

- Keep the samples as pristine as possible;
- Avoid any forward contamination, in case of a mission to Mars or other objects where there is the possibility of extant or extinct life.

One more point to be considered is the presence, or the possibility of presence, of infectious and potentially infectious materials. In this case, the samples are subject to strict national and international regulations. These regulations describe the proper use of packaging materials, as well as other shipping requirements.
Compliance with the rules will:

- Reduce the likelihood that packages will be damaged and leak;
- Reduce the exposures resulting in possible infections;
- Improve the efficiency of package delivery.

5.2 Key Points

D2.4 Main key points			
N.	**Key Point**	**Description**	**Notes**
K1	**Sample Holder Analysis**	Different archetypes of sample holders have been proposed and analyzed: • **Internal sample holder** (inside ESCF, restricted/unrestricted); • **Transportation container** (from ESCF to external laboratories and vice-versa, restricted/unrestricted); • **Special container** (from ESCF to external laboratories and vice-versa, restricted/unrestricted, for "special" samples).	
K2	**Transfer operations**	A sample transfer operation protocol has been studied, starting from some technological hypothesis regarding the sample manipulation infrastructure: the Sample Manipulation Cabinet (SMC)	
K3	**Pathogens and infective substances transfer**	This section applies to samples transportation to and from ESCF and external laboratories: • Regulations and standards • Packaging • Spill and clean-up procedures	

5.3 Recommendations

D2.4 Main recommendations			
N.	**Recommendation**	**Description**	**Notes**
R1	**Sample transportation**	The sample transfer should happen inside their canister or by means of specifically designed sample holder. The sample holders must be designed in order to be easily manipulated by both humans and a robotic arm.	
R2	**Environmental issues**	In any circumstances, the aim is to keep the samples always in a controlled atmosphere, to minimize the possibility of contamination. This applies also to external laboratories, where the architecture and the technical devices should be ready to continue the procedures.	
R3	**Materials and atmosphere**	The materials suitable to make the sample holder will be aluminium, compact gold sheets, Teflon, stainless steel, or quartz. The controlled atmosphere inside storage boxes and cabinets must be composed of inert gas, such as Argon or gaseous nitrogen (GN2).	**Table 5.1**
R4	**Sample holder sterilization**	All materials with which samples come into contact have to be sterilized, cleaned and packaged according to approved procedures, and introduced to the work area only through sterilized transfer locks.	
R5	**Internal sample holder function**	The internal sample holder is used to move the samples inside the ESCF. The internal sample holder should be designed as a small box that can contain samples in the diameter range from ≤100 □m up to few cm.	**Table 5.2**
R6	**Internal sample holder design**	The internal sample holder should be composed of a base and a cover. Once closed, it is sealed and able to isolate samples from the external environment. Bases should be available in 4 sizes. The same cover should be mounted on any of the four base sizes. The four sizes should have the same external interface, in order to mate with the handling system, the transportation container and the special container.	**Figure 5.1**
R7	**Transportation container**	The transportation container should be used to move the samples between the ESCF and the external locations. This is an external box where the internal sample holder should be located. We can assume that, for handling and safety reasons, the transportation container should house up to 8 internal sample holders.	
R8	**Transportation**	In case of restricted mission, when a bio-containment is	

	container for restricted mission	needed, the transportation container should be sealed.	
R9	**Special container function**	Special containers are samples holders embedded with an active control, to deliver special samples to external laboratories.	
R10	**Special container design**	The special container should be a sealed box, to maintain an inert atmosphere inside the internal sample holder, from curation to external locations. The special container should include a control unit to monitor the pressure inside the internal volume, and a gas reservoir to supply with additional amounts of inert gas, so to keep it constantly at a pressure greater than the external pressure. A double walled isolator should be designed in case of PP issues.	**Figure 5.2**
R11	**Sample manipulation cabinet**	A Sample Manipulation Cabinet (SMC) should be designed in order to extract the sample canister from the ERC and the samples from the canister, provide sample cataloguing and finally prepare the samples for their storage. The SMC should be a modular structure composed by a number of single modules, arranged in a row, with each module adjacent to the following and airtight doors separating them. This architecture should allow the sample to always stay inside a safe environment, minimizing the transfer phases and the risk of forward and backward contamination.	**Figure 5.3**
R12	**Sample operation flowchart**	See Figure 4	**Figure 5.4**
R13	**Pathogens and infective substances transfer**	There are a number of standards and regulations about this topic. Please refer to this literature.	

5.4 Methods

Not applicable

5.5 Tables and Figures

Table 5.1 *- Sample container materials pro & contra: three parameters have been considered (adsorption, stickness and interation time scale).*

Materials	Materials that regolith can adsorb	Stickiness	Interaction time scale
Aluminum	Al	Yes	months
Compact gold sheets	Au	Yes	months-years
Teflon	fluorocarbon fragments	No	months-years
Stainless steel	C	No	months-years
Quartz	Si and silicate ions	No	years

Table 5.2 *- Types of sample containers: there are four possible sample sizes for each type of sample holder/container.*

Function	Samples Sizes	Notes
Internal sample holder	- Up to 100 µm - From 100 µm 1 mm - From 1 mm to 1 cm - More than 1 cm	Samples retrieved from SRC or from external laboratories
Transportation Container	- Up to 100 µm - From 100 µm 1 mm - From 1 mm to 1 cm - More than 1 cm	To deliver sample to external laboratories
Special Container	- Up to 100 µm - From 100 µm 1 mm - From 1 mm to 1 cm - More than 1 cm	Samples holder embedded with an active control, to store and deliver special samples to external laboratories

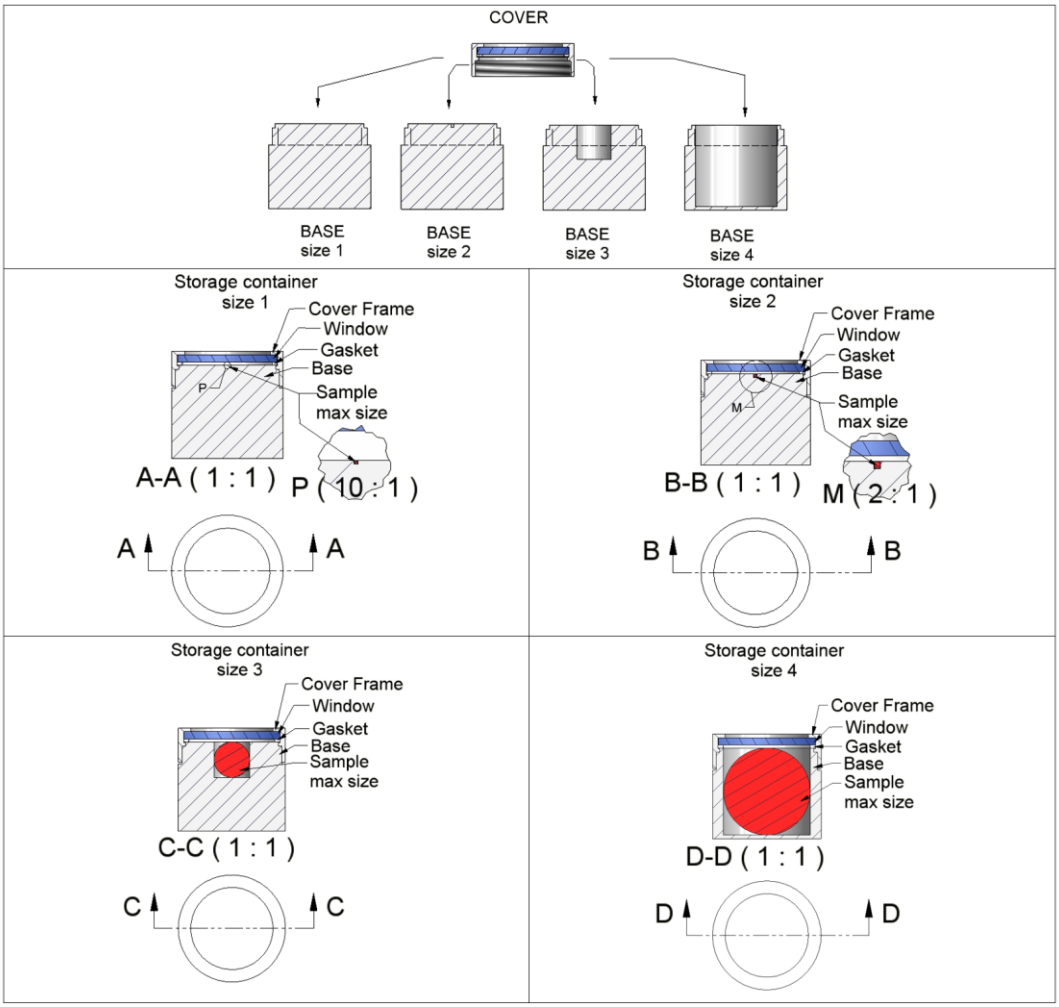


Figure 5.1 - *Possible design of the internal sample holder: four types of bases can be used according to the sample dimension, with a single cover; once closed, a window allows viewing of the sample inside the internal sample holder.*

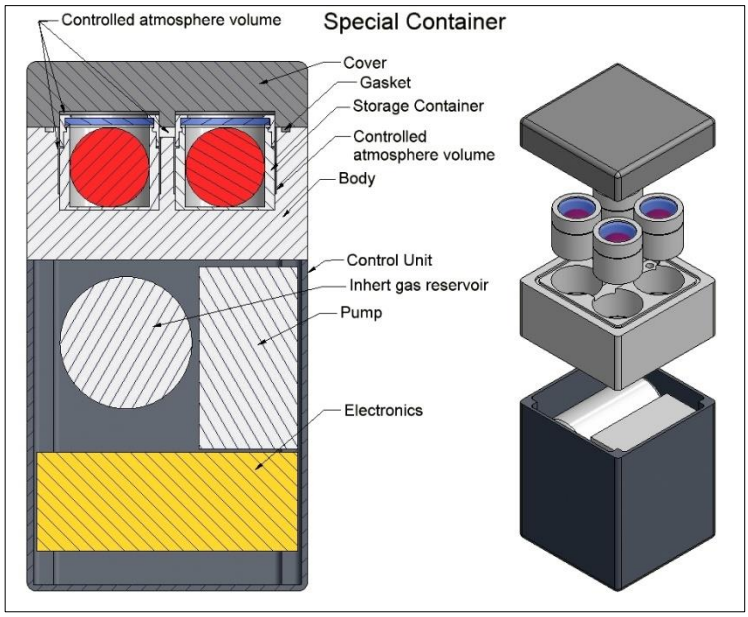


Figure 5.2 - *Possible design of a special container: the special container is able to contain a maximum of four internal sample holders and to maintain the right atmosphere, by means of a pump.*

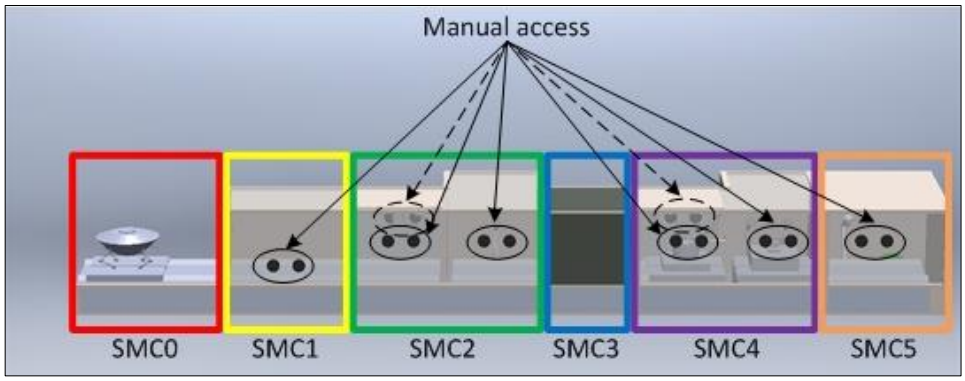


Figure 5.3 - *SMC design and modules in which it is subdivided: each sample manipulation cabinet (SMCx) allow a different operation: from the Earth Re-entry Capsule (SMC0) to the samples ready to be stored or delivered (SMC5).*

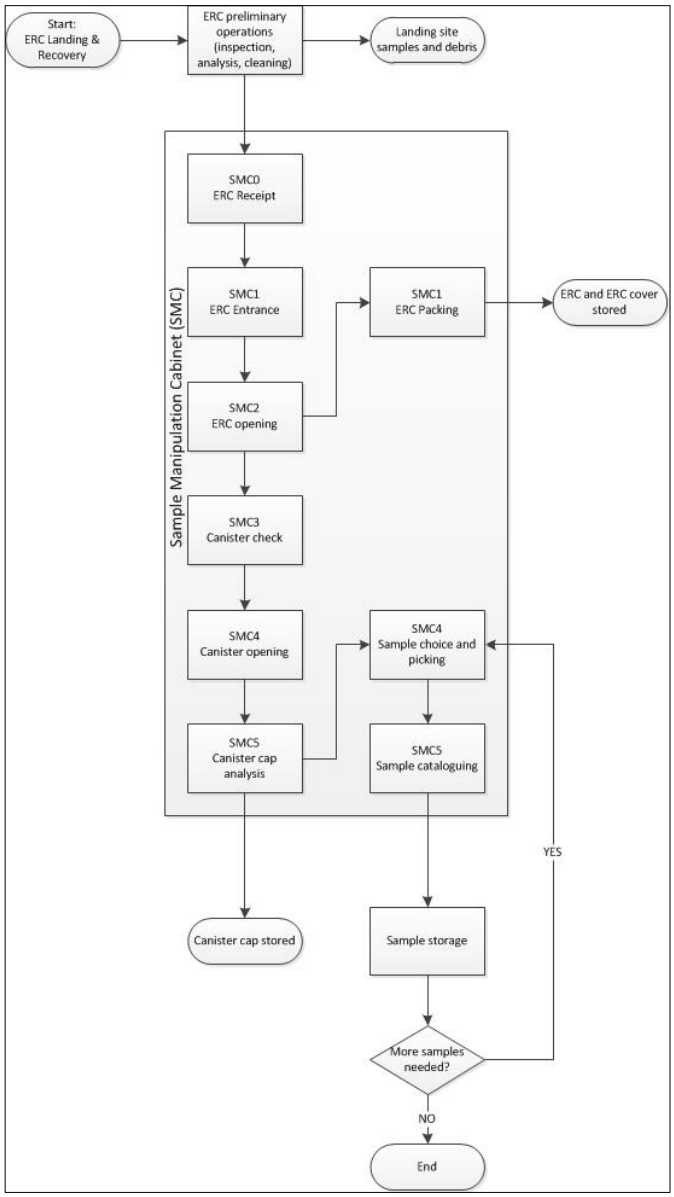


Figure 5.4 - *Flowchart of the main operations performed inside the Sample Manipulation Cabinet*

6 Facility requiremnts

6.1 Summary of D2.5

This section examines the current design specifications for high containment laboratories around the world and the testing regimes that are specified. It would be envisaged that certain sections of the sample return facility would be built to different specifications, which would reflect the use and need for containment within the section. For instance a section of the facility where restricted return samples will be examined would require to be built to or exceed current Biosafety guidelines of a biosafety level 4 (BSL4) facility in the host country and the EU Directive 2000/54. This is because it will not be known if the returned samplescontain any extraterrestrial lifeforms and under planetary protection guidelines, the facility must stop the release of an unsterilized particle. More specifically the probability of the release of a single unsterilized particle ≥0.2µm must be ≤1 x 10^{-6}.

Currently the highest level of containment is found in BSL4 laboratories, which are purpose built to handle to most dangerous pathogens on Earth. The facilities are designed to meet the criteria set by both national and international guidance documents. Aspects of the containment must be periodically tested to international and European standards, testing the facilities to these standards gives the knowledge to the operators that the facility is performing within their expectations. The validation and commissioning stage of the facility build should ensure that it is operating to the specifications of the original design brief. These specifications should meet and exceed the regulatory requirements for country where the SRF is built, to ensure the required level of protection for the workers and the environment. Validation should be completed by certified engineers and must be fully documented. Within the document redundancies for the specified areas are described in each section to highlight what needs to be considered during the design process.

6.2 Key Points

D2.5 Main key points			
N.	**Key Point**	**Description**	**Notes**
K1	**Facility technical requirements**	Brief overview of state-of-art of BSL4 facility: layouts, designs, planetary protection, working requirements, operating parameters.	
K2	**ESCF possible layouts**	To allow flexibility of the ESCF (in term of timeline and/or location) and to design some possible different layouts, a list of independent "high-level" units have been defined to produce different layouts.	**Method 5.4.1**
K3	**Facility construction and maintenance**	Overview of construction components and technical solutions to ensure that the facility operates effectively to eliminate the likelihood for the release of infectious particles from either the primary or the secondary containment: construction materials, build finishes, pressure requirements.	
K4	**Personal protection measures**	Technical requirements to ensure the highest level of protection to the facility workers have been defined and reported.	
K7	**Waste handling**	The facility produces waste from a number of different sources, which need to be treated to reduce any potential contamination to an appropriate level. An overview of legislation, waste handling and management is reported.	
K8	**Security**	A study of the different approaches to maintain the security of the facility, staff, samples and the information generated.	
K9	**Communication**	The communication of the net layout, inside the facility and to the media, has been analyzed.	
K10	**Processing requirements**	A graphical representation of the sample procedures inside the facility for sample has been proposed using a swim-lane flow-chart approach.	**Figure 5.4**
K11	**Worklow of samples and number of samples**	All the activities performed inside the facility require a planetary protection assessment. The major issues arises from the activities performed on the samples. Thus, an estimation of the number of operations and the amount of flows is shown.	**Method 5.4.2 and 5.4.3**

6.3 Recommendations

D2.5 Main recommendations			
N.	**Recommendation**	**Description**	**Notes**
R1	**Facility Layout**	At least four possible scenarios can be defined. Actually, starting from the main pros and cons regarding costs, political issues, personnel training, redundancy, etc., it is not possible to identify the best option.	**Method 6.4.1**
R2	**Planetary protection requirements**	According to the state-of-art, within the sections where restricted return samples are handled, there is a requirement to maintain sterility and also to ensure that the probability of the release of a single unsterilized particle ≥0.2µm must be ≤1 x 10^{-6}	
R3	**Facility construction**	The main units of the facility should be sealed boxes that have a limited number of controlled leak paths through its fabric For a facility that will be receiving restricted return samples, then the construction would need to be similar if not more stringent than that of existing BSL4 laboratories to ensure there was no release	
R4	**Facility future adaptation**	The facility should be built to allow for adaption of use in the future (future proofing). This requires provision of space and other utilities in the design and construction allowing equipment to be incorporated into the laboratories.	
R5	**Construction issues**	A brand new facility approach should be preferable, since the retrofitting of an existing building is difficult and extremely costly in comparison to the design and building of a new dedicated facility.	
R6	**Build finishes**	The surfaces should be made with a smooth finish, be resistant to water and chemicals used in the disinfectant. 90° angles should be avoided. The material should be unaffected by the effect of the pressure differential. Doors should be able to maintain the pressure gradient either using manual latches or automatic pneumatic seals. Regular inspections of all the finishes are required.	
R7	**Biological safety cabinets**	The European standard (BS EN 12469) should be followed, to provide the testing regime and methods to ensure a BSC III is operating to a safe level.	
R8	**Positive pressure suits**	The British standard BS EN 464:1994 is currently suggested for pressure testing of suits used in high	

		containment.	
R9	**HEPA filters**	Testing of HEPA filters should be completed using the British standard 6609:2007 and/or the USA NSF/ANSI Standard 49-2007.	
R10	**Leak emergency procedures**	A risk assessment should be performed prior to the commencement of work to identify what emergency steps should be taken for each scenario envisaged. If a leak is detected, an assessment should be completed as to the impact of this release. Plans of action should be drawn up, (e.g. appropriate PPE required for the workers. The most effective decontaminant to be used, etc.). These can then be inputted into the risk assessments and emergency procedures produced.	
R11	**Waste treatments**	Within the facility, hazardous waste should be collected as soon as possible once it is produced, treated in order to reduce the amount and risk, stored in a safe area and then disposed of.	
R12	**Security**	Physical security elements are required in order to prevent unauthorized access and protect the samples from removal for non-official purposes. They can be site specific and depend on the location of the facility. Physical security around the site should include at least robust security perimeter fencing, 24 hour operated security and a number of different layers of access control to the laboratories. The infrastructural security should follow a concentric approach.	**Figure 6.1**
R13	**Communication net inside the facility**	A communication plan is mandatory: personnel should be aware of the plan and should know what to expect and what is expected of them. The more sensible are the activities performed inside the facility units (i.e. the SRF and SCF), the more redundant and self-powered the communication devices should be. An plan should be operative, in case of emergency: redundant systems are mandatory in case of unexpected incidents when an emergency affects the ESCF or the surrounding environment.	**Figures 6.2, 6.3**

6.4 Methods

6.4.1 ESCF possible layouts

To allow flexibility of the ESCF (in term of timeline and/or location) and to design some possible different layouts we have defined a list of independent "high-level" units each fulfilling a unique function, as in the following table.

UNITS	Main activities
PRF RESTRICTED	**Portable Receiving Facility Restricted** - Assessing, cleaning and packaging he spacecraft on the landing site. Delivery of the spacecraft to SRF.
PRF UNRESTRICTED	**Portable Receiving Facility Unrestricted** - Assessing, cleaning and packaging the spacecraft on the landing site. Delivery of the spacecraft to SRF.
SRF RESTRICTED	**Samples Receiving Facility Restricted** - Receiving the sample container, cleaning & opening of the outer layers and delivery of the unopened sample canisters to the curation facility. Clean and high containment environment.
SRF UNRESTRICTED	1. **Samples Receiving Facility Unrestricted** - Receiving of the sample container, cleaning & opening of the outer layers and dissemination of the unopened sample canisters to the curation facility. Clean environment.
SCF RESTRICTED	2. **Samples Curation Facility Restricted** - Receiving of the sample canister, accessing the sample Life Detection (including Biohazard Assessment), Curation. Clean and high containment environment.
SCF UNRESTRICTED	**Samples Curation Facility Unrestricted** - Receiving of the sample canister, accessing the sample. Curation, Dissemination to science labs. Clean environment.
WORK SPACE	**Work Space** - Offices, meeting rooms, social rooms, restaurant.
PUBLIC OUTREACH	**Public Outreach** - Museum, exhibition area.

ANALOGUE FACILITY	**Analogue Facility** - Semi-mirror facility for personnel training, instruments and protocols testing on analogue samples. For potentially biohazardous samples.
REMOTE STORAGE	**Remote Storage Facility Restricted** - Storage under dead-mode of a TBD part of the potentially biohazardous samples. Clean and contained conditions.
REMOTE STORAGE	**Remote Storage Facility Unrestricted** - Storage under dead-mode of a TBD part of non biohazardous samples. Clean conditions.

Since units are independent from one another, they can be built in different locations, and/or over time.

We consider four possible scenarios. Right now, it is not possible to identify the best option between the different layout, since many pros and cons can be defined, regarding costs, political issues, personnel training, redundancy, etc. The main pros and cons are highlighted for each possible layout, as follows.

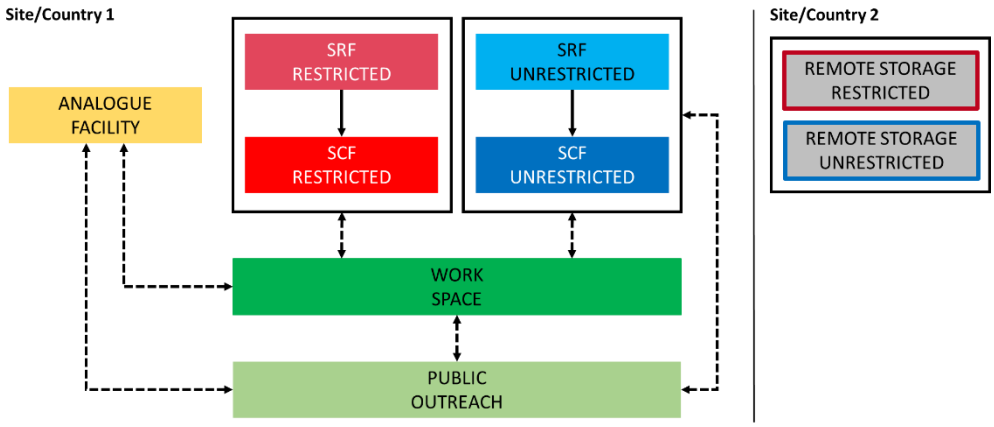
It has to be remarked that the Portable Receiving Facility (PRF) is not shown in the diagrams, since it will be installed on the landing site, if necessary.

- **Integrated approach**

  All units (except for remote storage) are built on the same site, not necessarily at the same time.

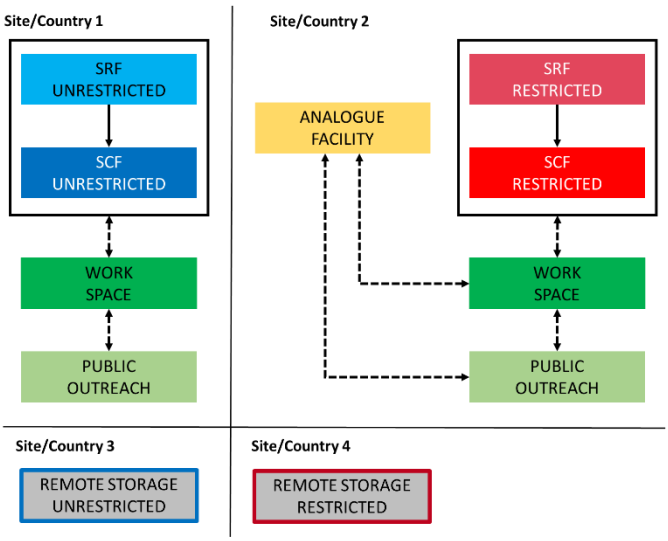
  Pros: the entire ESCF will be built in one location, requiring a unique working team. This layout will be less expensive.

  Cons: if shut down happens because of a catastrophe or for any other reason, all activities will be over. Political issues because the samples will reside in the same country. There is a need of a site large enough to build the entire structure.

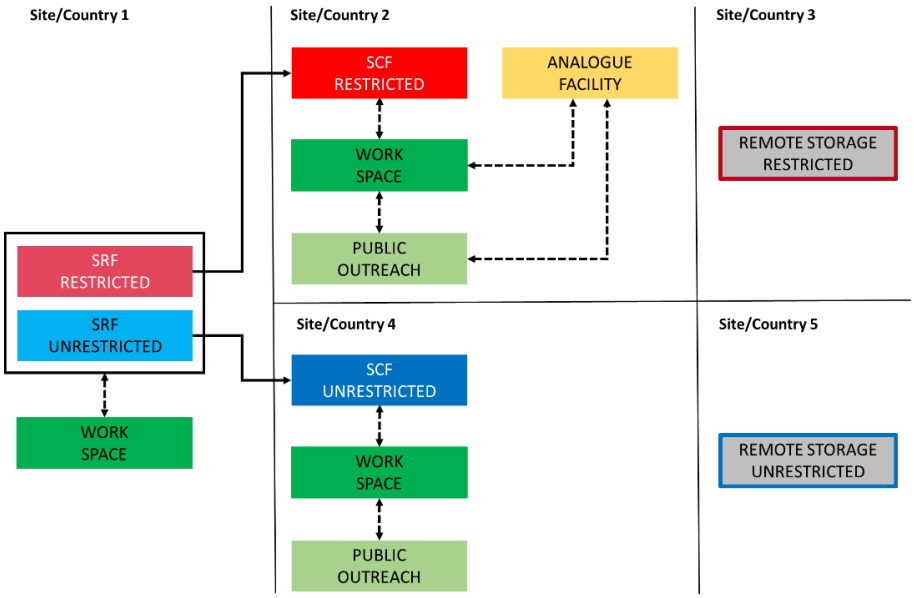


- **Unrestricted vs. Restricted Separated Sites**

Biohazardous samples and non-biohazardous samples are treated independently, with SRF/SCF built on different sites.
Pros: more than one country involved. Redundancy. Smaller initial sites.
Cons: training and skills of workers less easily transferable between restricted and unrestricted. The cost will be higher than for the integrated approach.



- **Common receiving facility – Separated curation facilities**
  All missions are received in the same place and samples are then shipped to distinct curation facilities.
  Pros: the receiving facility is used more often, to counterweight the transient function of it. More partners involved. Redundancy. Smaller initial sites.
  Cons: high replication of workers and working space. Training and skills of workers less easily transferable between restricted and unrestricted.

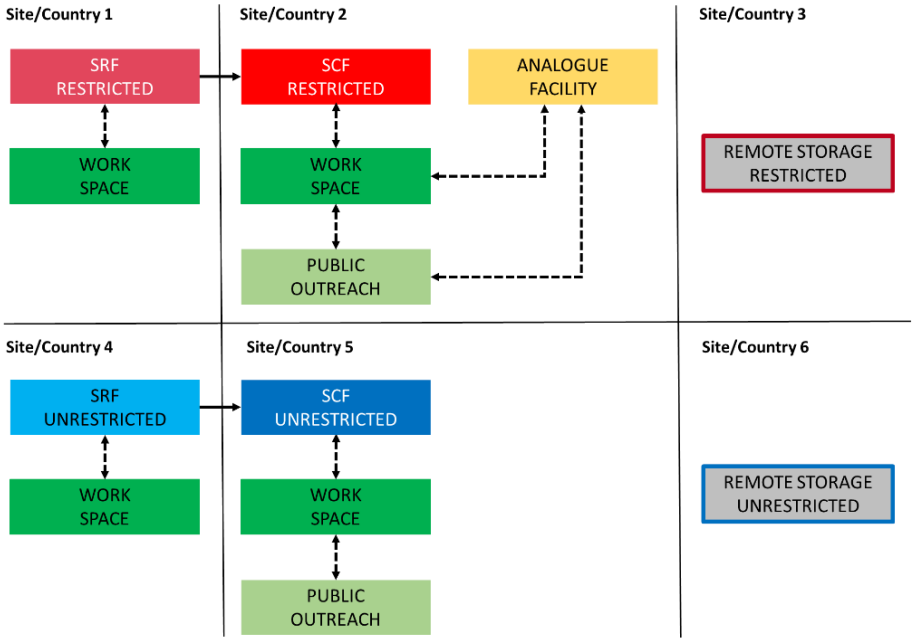


- **Distributed approach**

  All functions (receiving and curation) are scattered in different locations.

  Pros: More partners involved.

  Cons: Very high replication of workers and working space. Costs. High risks of transports between facilities.



6.4.2 Workflow of samples

The total amount of samples available (TS) for the operations can be divided in two groups:

- Samples to be preserved for undefined amount of time: PS
- Samples to be analyzed for LD/BH: AS

Where, obviously:

TS = PS + AS

In some cases PS = 0, when the total amount is lower than a defined value and all the samples requires to be analyzed. For example, regarding the amount of samples subjected to LD/BH assessment, Rummel *et al.* in 2002 proposed a 10% as a rough estimate of a reasonable amount of sample to be used. Furthermore, MEPAG E2E-iSAG suggested that for planetary protection aspects a mass of 1.5 g of each sample should be assumed reasonable amount to conduct LD and BH tests. According to this suggestion, we can assume that if TS < 1.5g, it follows that all the samples must be analysed: TS = AS and PS = 0.

Due to LD/BH assessment, some of the samples may be subjected to destructive analyses and consequently lost (LS).

Depending on the results of LD/BH assessment, the part of AS samples not subjected to destructive analyses, can be devised in two groups:

- Samples to be sterilized: SS
- Samples to be catalogued: CS

There are three possibilities:

- LD/BH assessment is negative, and then all the analyzed samples are now sent to the cataloguing operations:
  AS = CS +LS and SS = 0
- LD/BH assessment is positive, but for some reasons not all the analyzed samples must be sterilized:
  AS = SS + CS + LS
- LD/BH assessment is positive, and all the analyzed samples must be sterilized:
  AS = SS + LS

In the last two cases, once sterilized, all the sterilized samples (SS) are then sent to the cataloguing operations (SCS), so:

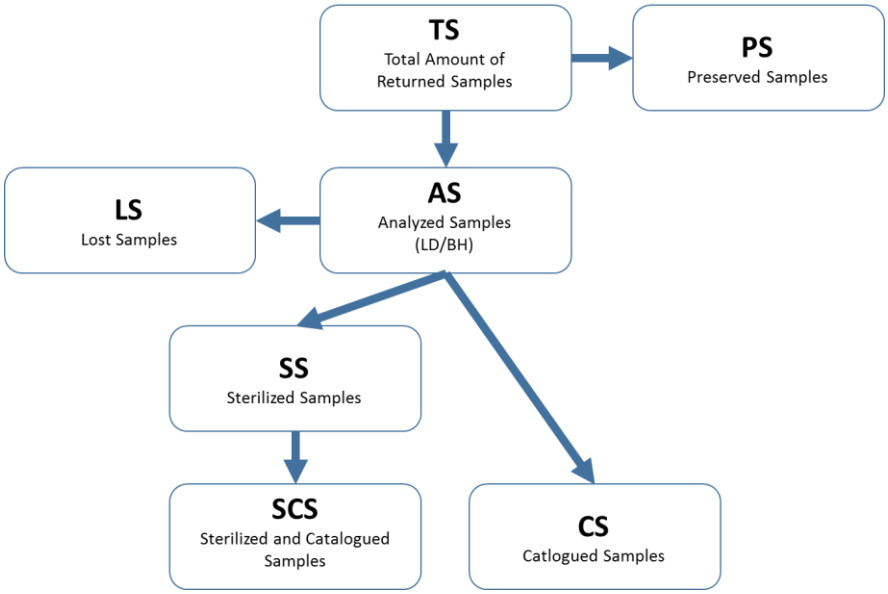
SS = SCS

The difference between the CS (catalogued samples) and SCS (sterilized and catalogued samples), is that the SCS are sterilized before being catalogued.

Resuming, in the end there are the following possibilities:

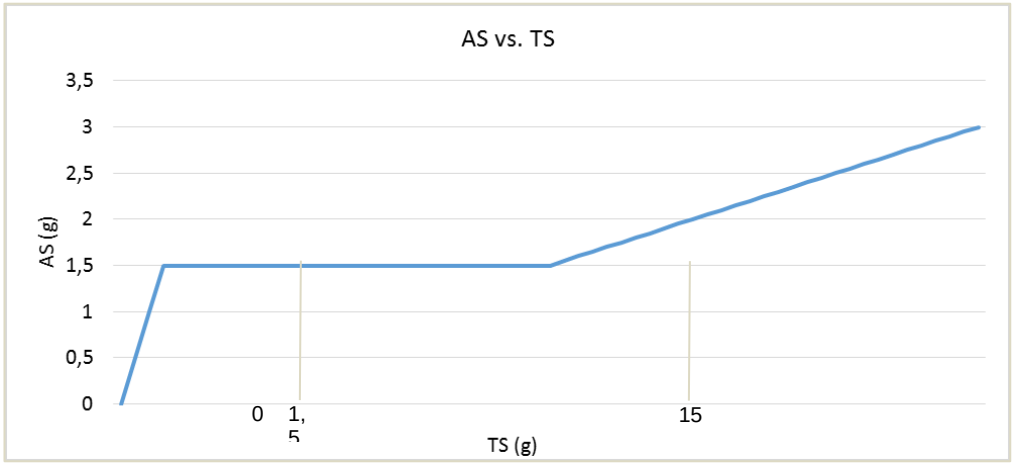
- LD/BH assessment is negative:
  TS = PS + CS + LS
- LD/BH assessment is positive, but for some reasons not all the analyzed samples must be sterilized
  TS = PS + SS + CS + LS (where SS=SCS)
- LD/BH assessment is positive, and all the samples must be sterilized:
  TS = PS + SS + LS (where S=SCS)

The entire flow should be resumed as in the following figure:



6.4.3 Number of samples

Here again we have to focus on the PP activities, so starting from AS and the following operations. As mentioned in the previous paragraph, if TS < 1.5g, all the samples have to be subjected to LD/BH assessment. In case of a larger amount of available returned samples AS = 0.1TS. It is possible to resume those suggestions in the following figure, where the amount of AS versus the TS is shown.



The AS have to be subjected to LD/BH assessment, The amount of sub-sample subjected to LD/BH is the result of the technique sensitivity, fraction of variable observable (molecule, polymer, cell, etc.), number of analysis repetition.

This implies that at this stage of the study it is impossible to forecast the actual techniques needed to assess LD/BH. Our goal became to provide a set of formula able to calculate amount of samples and flows, easily convertible in actual numbers once some coefficients are known.
The first step is to define an ideal sample quantity defined as "*single sample*" (s_i), independent from its state: solid, liquid, etc. The single sample is only defined by its mass (m_i) and volume (v_i). The latter are intrinsic or defined by the operation (e.g. when a returned sample is cut to prepare it to an experiment).
The total number of single sample subjected to LD/BH assessment is N, and:

$$M_{tot} = \sum_{i=1}^{N} m_i \quad V_{tot} = \sum_{i=1}^{N} v_i$$

Any time an instrument is used, the following parameters have to be taken into account:

- The repetitions, the number of experiments we need to repeat on the same instrument, to allow the instrument to produce an actual result, defined by the *number of repetitions* (R).
- The instrument sensitivity, the smallest absolute amount of change that can be detected by a measurement. The sensitivity can be traduced as the lowest quantity of samples needed to allow the instrument to produce an actual result, defined by the *number of single samples needed* (N_S).

It has to be remarked that, at this stage of the study, is not useful to provide an actual value for the sensitivity of each instrument, since this value depends on the specific chosen instrument.
For each instrument, once the experiment setup it is defined, the amount of samples needed (M_{INS} and V_{INS}) is given by:

$$M_{INS} = \sum_{j=1}^{R} . \sum_{i=1}^{N_S} m_i \quad V_{INS} = \sum_{j=1}^{R} . \sum_{i=1}^{N_S} v_i$$

Where:

- Ns = 1 if the quantity of the single sample is greater or equal to the minimum quantity detectable by the instrument,
- Ns > 1 if the quantity of the single sample is lower than the minimum quantity detectable by the instrument.

Once the instruments for LD/BH are chosen, the total amount of samples (in mass or volume) can be calculated as the the sum of M_{INS} and V_{INS} obtained from each instrument.

6.5 Tables and Figures

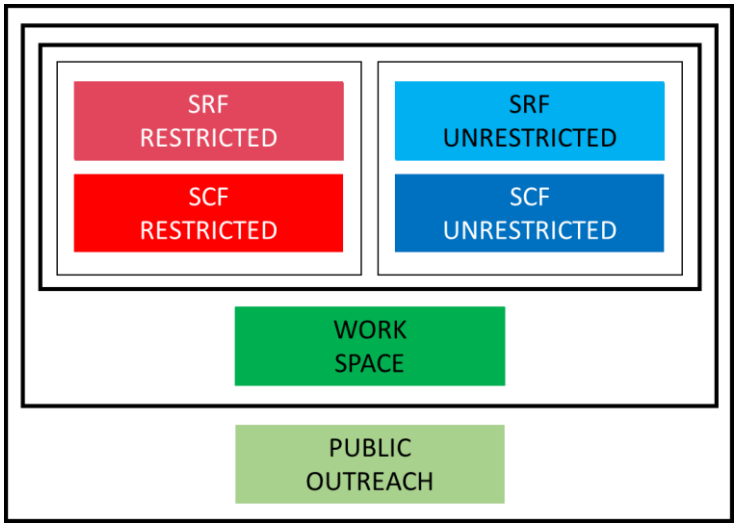


Figure 6.1 - *Facility concentric layout: each black rectangle define a physical security barrier.*

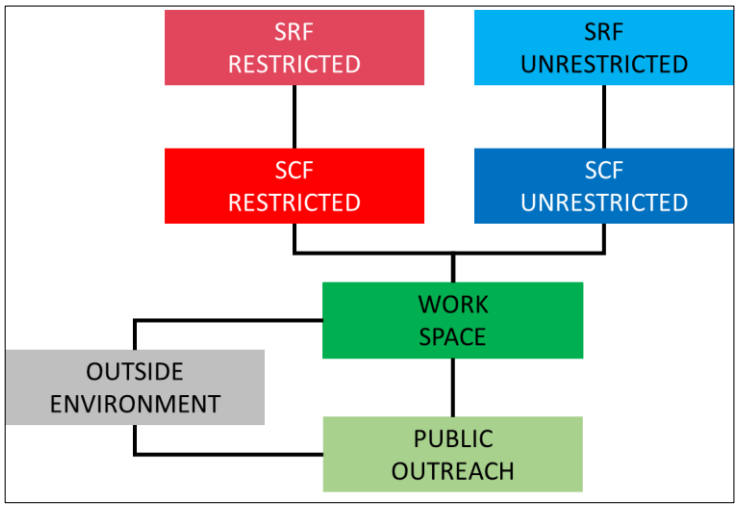


Figure 6.2 - *Facility standard communication net*

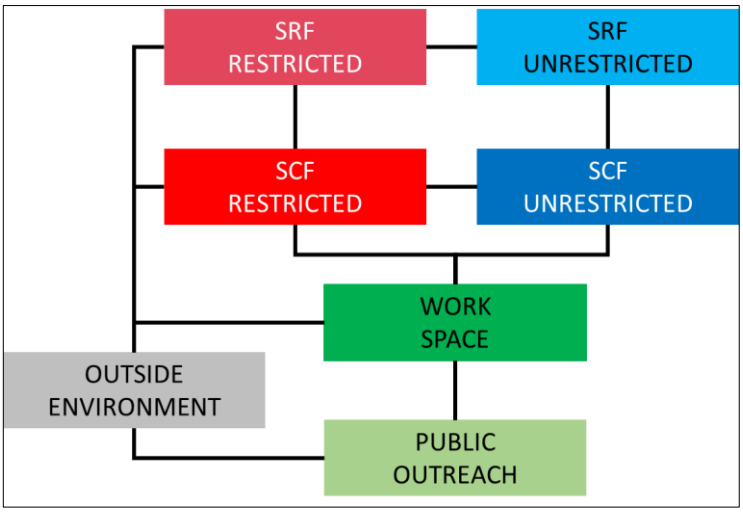


Figure 6.3 - *Facility emergency communication net*

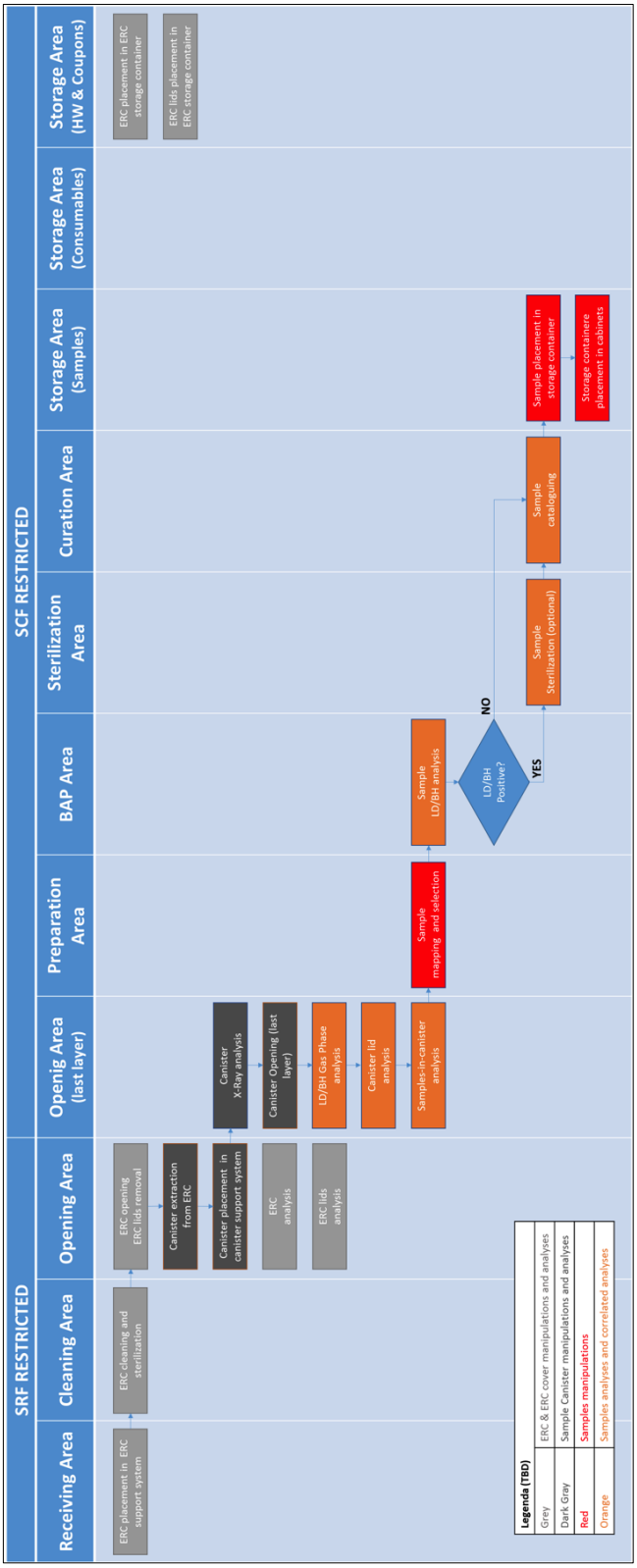


Figure 6.4 - *Swim-lane flow chart showing the samples procedures inside the units (in blue): each procedure has a different color, according to the type (see legenda inside the figure).*