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

FACILITY REQUIREMENTS

WP 2 D2.5

Issue 1 Rev. 1

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1. Introduction

This document will examine 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 samples returned contain any extraterrestrial lifeforms and under planetary protection guidelines the facility must stop the release of an unsterilised particle. More specifically the probability of the release of a single unsterilised particle ≥0.2µm must be ≤1 x 10^{-6}[1]. 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 [2]. 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 [3]. Within the document redundancies for the specified areas are described in each section to highlight what needs to be considered during the design process.

2. Technical requirements for structure, environments, waste handling, security, and communications

2.2 Structure

The high containment laboratory is usually a small component of the entire research facility. The USA produced BMBL requires that a BSL4 laboratory is either a separate building or a clearly defined and isolated zone within an existing facility [4]. A number of different approaches can be taken for the location of a BSL4 suite in a research facility. The BSL4 suite can either be:

- **Incorporated into the research facility**: this approach is taken by a number institutes worldwide e.g. at PHE Porton, UK, and the US Army Medical Research Institute of Infectious Diseases (USAMRIID), Maryland, USA.
- **Separate to the existing research facility building**: e.g. the P4 Jean Mérieux BSL4 facility, Lyon, France.
- **Adjacent to the other research facilities**: e.g. the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada.

Each of these approaches allows the BSL4 to maintain a high level of security and safety, with the selection of the laboratory location dependent on the requirements identified during the design stages. The current designs of high containment facilities are discussed in the following sections.

2.3 Biosafety level 4 (BSL4) facilities

The highest level of containment laboratory that is available for the handling of biological samples in is a BSL4 laboratory. These laboratories are designed to allow the safe handling and storage of samples, without exposing the workers or surrounding environment to the hazards. The laboratories are usually designed to provide containment for the specific activities that will be undertaken within them e.g. diagnostics or research.
There are two different types of BSL4 facility used around the world. These are differentiated by the type of containment that is used within the laboratories, either cabinet lines or positive pressure suits. Cabinet line laboratories are only found within the UK whereas suited laboratories are used around the world [3, 4]. However, for purely diagnostic work, cabinet lines are becoming more attractive such as in Georgia State University, US.

2.3.1 Suited laboratories

Every person entering the laboratory is required to wear a positive pressure suit connected to a breathing air supply, which is required to inflate the suit. The suit's material and pressurization provides protection to the operator from aerosolised and droplet contamination. As a general rule all procedures carried out using infectious material is performed within an open fronted safety cabinet (biological safety cabinet (BSC) class II). The worker will be required to use a chemical shower on exit of the facility prior to removing the suit and then a further personal shower (Figure 1).

Figure 1. Worker within a positive pressure suit (Picture credit PHE).

2.3.2 Cabinet line laboratories

All infectious material is handled within interconnected class III BSCs, otherwise known as glove boxes. These cabinet are fully sealable and operate at a high negative pressure (<-200Pa). A spine of cabinets forms the basis of the cabinet line, with cabinets branching off from the spine where the work is performed (Figure 2). The cabinet spine is generally operated at a lower negative pressure from the cabinet branches so air is drawn away from where the work is being completed. This set up allows all equipment to be contained within the cabinet line. One end of the cabinet line will be connected to a double-sided autoclave, where all waste will be sterilized on leaving. The workers must wear disposable clothing within the laboratory and on exit are required to use a personal shower.

Figure 2. A cabinet line laboratory. Image credit PHE

2.3.3 Facility layouts

High containment facilities utilise a negative pressure gradient to reduce the likelihood that an infectious particle will be released from the laboratory areas. Work is undertaken in primary containment that is at a negative pressure to the laboratory, the laboratory is at a reduced pressure to the shower or changing room, and so on. Each area will be at a reduced pressure to the environment outside of the laboratory facility.

Figures 3 and 4 show the basic design of cabinet line and suited BSL4 laboratories. Figure 5 shows a more detailed schematic of the BSL4 suited laboratory facility in Galveston Texas, with the different rooms indicated. In this figure, the areas in blue are 'clean' and the darker orange colour represents contaminated areas.

Figure 3. The typical design of a cabinet line BSL4 laboratory [3]

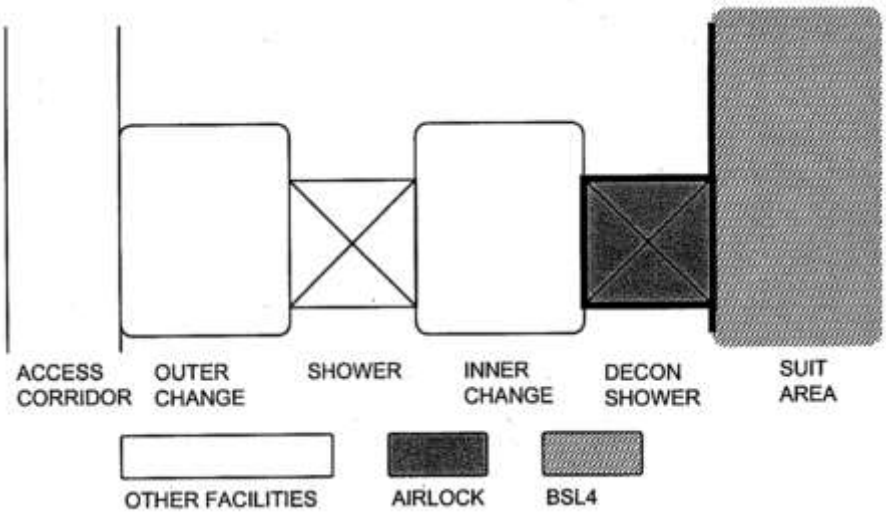

Figure 4. The typical design of a suited BSL4 laboratory [5]

Figure 5. A schematic plan of the University of Texas Medical Branch BSL4 laboratory. Picture credit University of Texas Medical Branch.

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 (Table 1).
A "low level" set of units, more detailed, will be presented in the following paragraphs, when, for high level units, planetary protection issues occurs.

Table 1 – ESCF main units	
UNIT	**Main activities**
PRF UNRESTRICTED	Assessing, cleaning and packaging the spacecraft on the landing site. Delivery of the spacecraft to SRF.
PRF RESTRICTED	Assessing, cleaning and packaging he spacecraft on the landing site. Delivery of the spacecraft to SRF.
SRF 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	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	Receiving of the sample canister, accessing the sample Life Detection (including Biohazard Assessment), Curation. Clean and high containment environment.
SCF UNRESTRICTED	Receiving of the sample canister, accessing the sample. Curation, Dissemination to science labs. Clean environment.
WORK SPACE	Offices, meeting rooms, social rooms, restaurant.
PUBLIC OUTREACH	Museum, exhibition area.
ANALOGUE FACILITY	Semi-mirror facility for personnel training, instruments and protocols testing on analogue samples. For potentially biohazardous samples.
REMOTE STORAGE	Storage under dead-mode of a TBD part of the potentially biohazardous samples. Clean and contained conditions.
REMOTE STORAGE	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.
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.
We consider four possible scenarios. Right now, it is not possible to identify the best option between the different layouts, 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.

- **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 single 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 a site large enough to build the entire structure.

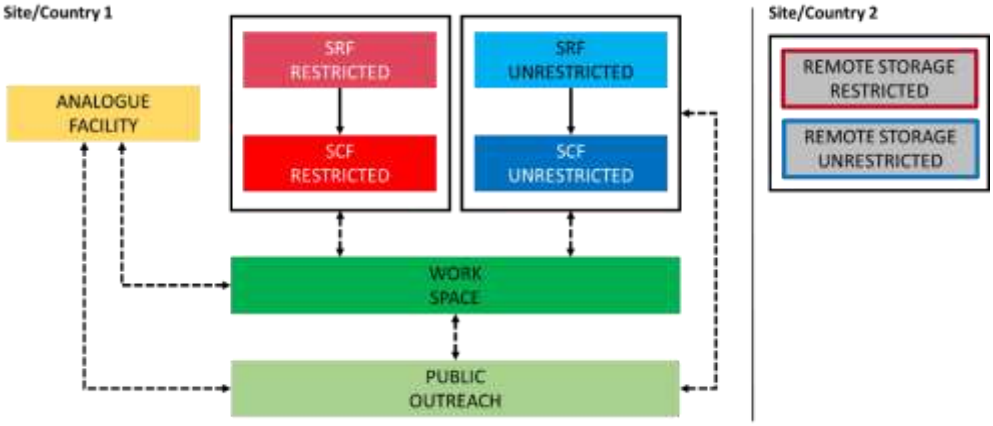

Figure 6. Integrated approach layout

- **Unrestricted vs. Restricted**
  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.

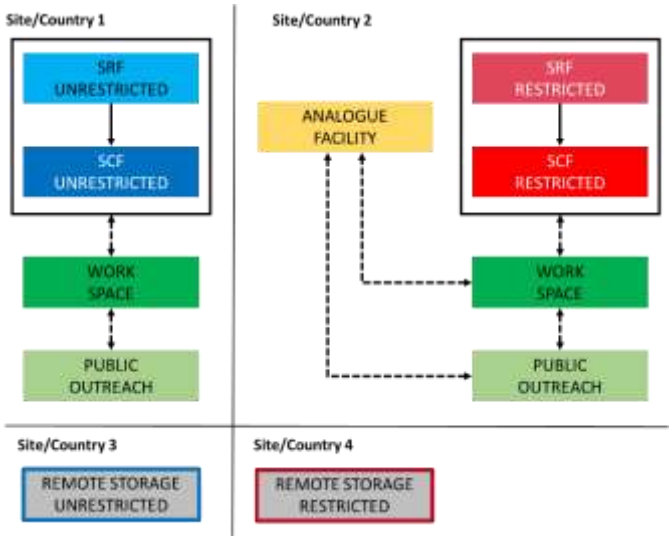

Figure 7. Unrestricted vs. Restricted layout

- **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.

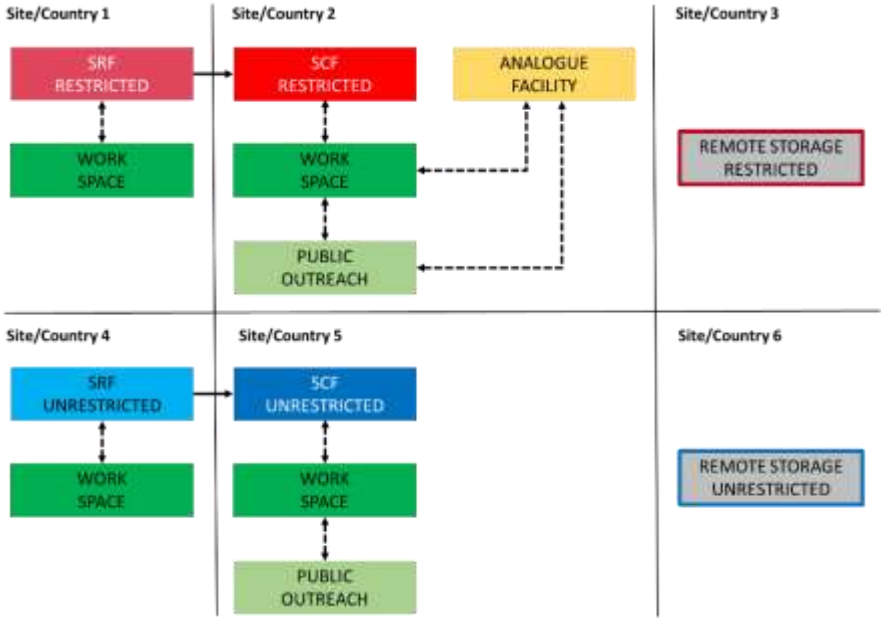

Figure 8. Common receiving facility/Separated curation facilities layout

- **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 transport between facilities.

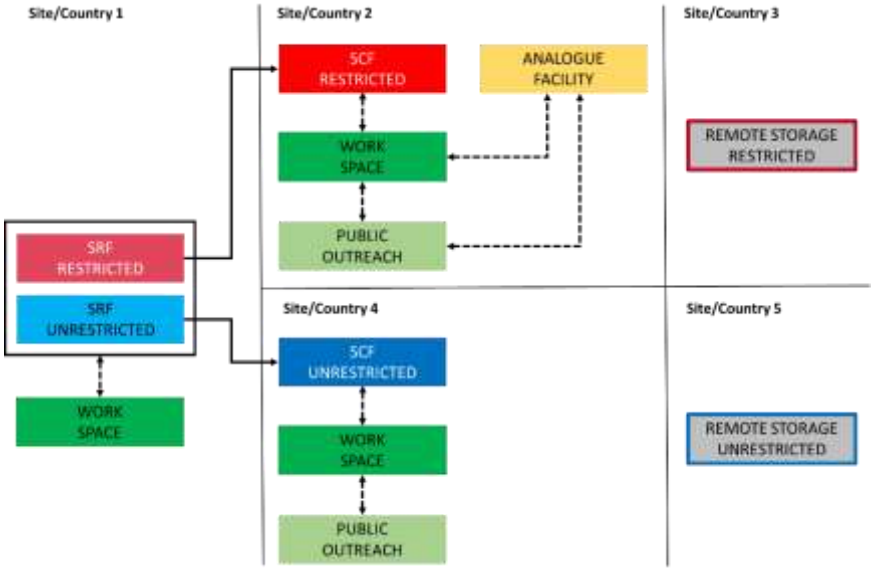

Figure 9. Distributed approachlayout

2.3.4 Planetary protection requirements

The sample receiving and curation facility will be required to ensure that it can maintain a pristine environment as much as practicable to ensure sample sterility and protection to the workers and the external environment. In the sections of the facility that handle the unrestricted return samples there will only be the requirement to maintain sterility within the working areas where the samples are manipulated and tested. Within the sections where restricted return samples are handled then there is a requirement to maintain sterility and also to ensure that the probability of the release of a single unsterilised particle ≥0.2μm must be ≤1 x 10-6 [1].

2.3.5 Working requirements and operating parameters

There will be a number of different working requirements that need to be identified based on what work is being undertaken there, for example the room's pressure differentials will vary, with a greater negative pressure required in a laboratory handling restricted return samples compared to the entry room.
Different pieces of equipment will require different parameters to be recorded prior to use to ensure they are operating correctly. A biological safety cabinet will have a log sheet attached to it where the operations performed in it and decontamination process that has been used

can be recorded, this will be checked and signed by each subsequent user to maintain a safe operating environment.
The key operating parameters for each section will need to be determined and set in an identification sheet that will be checked and monitored before each use of a piece of equipment, or if required on a regular basis (i.e. each day).

2.4 Construction and maintenance

A BSL4 facility requires a number of different construction components to ensure it operates effectively to eliminate the likelihood for the release of infectious particles from either the primary or secondary containment. The main idea of the construction of the BSL4 laboratory is to build a sealed box that has a limited number of controlled leak paths through its fabric. Engineering designs are used for the ventilation of the laboratory creating both a negative pressure and inflow of air. Facilities are designed and built with the knowledge of what procedures will be undertaken within them: for example in BSL4 animal facilities a necropsy laboratory will have the most negative pressure because this is an area where there is a high risk of aerosolisation of the infectious agent and maintaining the room under the most negative pressure will help to eliminate the release of aerosols if they occur.
Facilities can also 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.
The construction of the facility will again depend on the requirements identified. 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.

2.4.1 Construction materials

There are a number of different construction materials used in facilities around the world. Two examples of different construction materials are the P4 Jean Merieux facility in Lyon, France and the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada. The P4 facility in France was constructed by straddling and existing laboratory building, Because of this it was not practical to use concrete walls for the shell and as such an alternative technology was used. Steel faced urethane panels (five inches thick) were used to form the barrier. The panels were joined using cam action locks and silicon sealant used to ensure an airtight seal between them. The panels are usually used for building environmental rooms and proved to be rapidly put together, cost effective and stable building material [5]. Each area within the laboratory suite is connected by doors that utilise seals, which inflate with compressed gas to create a hermitic seal. The doors stay closed using magnetic locks, which are interlinked to ensure that adjacent doors are not opened at the same time. The facility's shell is not pressure tested [5].
The Canadian BSL4 facility is constructed from concrete, which has a tendency to crack and shrink over extended periods of time affecting the air tightness of the facility, leading to the requirement that regular inspection and maintenance of the facility is required to ensure structural integrity is maintained. To overcome this, a special formula concrete for the

Canadian facility was used and poured early in the construction to reduce cracking and shrinkage. This has now become common practice, especially with facilities in the USA [5]. The construction of a high containment laboratory within a pre-existing facility may not use the construction materials within that facility. If the space was appropriate then the laboratory could be constructed within the facility using a more modular approach. There would be major difficulties with this approach, such as providing suitable air handling and effluent treatment areas. Issues would also be seen trying to manufacture pressure gradients between areas of the laboratory suite and providing utilities such as water and compressed airlines. This would make the retrofitting of an existing building difficult and extremely costly in comparison to the design and building of a new dedicated facility. It is also important to consider whether the fabric of the building will be able to withstand the pressure set points that are stated in the design requirements.

2.4.2 Negative Pressure

The EU Directive 2000/54 and other national regulations state that there is a requirement for negative pressure within the laboratory but the set points that are required vary greatly between regulative documents [2]. The negative pressure of a laboratory will cause directional airflow within the laboratory, moving from areas that are less hazardous to areas with greater hazards. The level of the pressure differentials should be stated as a design requirement. The UK regulatory body the Health and Safety Executive (HSE) have in their guidance stated: "There are no legally specified pressure differentials and guidance values can vary but are approximately -30 Pascals (30 Pa) for each layer of the pressure cascade (ACDP) or (for DEFRA) at not less than -75 Pa total difference between the laboratory and ambient pressure" [3]. There needs to be a balance when the pressure differentials are selected for the facility as whilst high differentials can afford a high level of protection the fabric of the building may be damaged and it can make opening doors between sections difficult.

In other countries facilities use a range of pressure differentials such as:

- The BSL4 laboratories in Winnipeg, Canada, use a series of four airlocks that have a difference of 50 Pascals between them [5].
- The National Institute of Health (NIH), USA, employs a pressure differential system of -50 Pascals within the facility shell, then further -12 Pascals in the suit entry and laboratory areas [5].
- The P4 BSL4 facility in Lyon, France, uses a gradient of -40 Pascals in the entry room, reducing to -90 Pascals in the animal facility autoclave room [6].

To maintain the supply of the negative pressure and inward flow of air to the facility there should be at least two exhaust fans and one supply fan. The exhaust fans can either be run singularly and in the event of a fan failure the other automatically starts to maintain negative pressure, or both fans are run at 50% maximum and then can be increased with a single fan failure. It will also be required that the inlet and exhaust fan units are interlocked as this will stop the loss of negative pressure if the exhaust fans fail. There should be appropriate

monitoring devices to ensure the correct pressure is maintained. These monitoring devices will also help workers and engineers to ensure the requirements set within the design are maintained. Documentation records should be kept to demonstrate that the facility has been operating correctly.

2.4.3 Build finishes

The surfaces used within a BSL4 facility should be made with a smooth finish and be resistant to water (e.g., walls, floors and benches). This is because the transportation of a liquid through a surface can lead to the penetration of a pathogenic organism. These surfaces should also be resistant to the chemicals used in the disinfectant process in the facility, this will also facilitate easy cleaning of the surfaces. The construction of the floors should not give any 90° angles, rather smooth bends that allow for easier cleaning of the floor and prevents any pooling of liquids on the floor. The external shell of the facility should be constructed to restrict the ingress of animals or insects.
The walls of the laboratory, and any part of the facility that is under pressure, must be constructed to remain unaffected by the effects of the pressure differential, this includes the gaskets and seals used within. The walls must be sealed not only for pressure tightness but also to contain any fumigant used within them. Regular inspection of the walls will allow the identification of any damage to them and permit their rapid repair. In the UK the HSE have generated a list of the materials they have recommended for the finish of the walls in a high containment facility, they are: epoxy or polyester coated plaster, and rubberised paints [3].
The US BMBL advises against having external windows into a high containment laboratory itself, but if there are present then they must be non-opening and break-resistant [4].
It is important that when designing the facility the transit spaces and doors are large enough to permit the transport of equipment through them and also consideration to 'future proofing' where any equipment potentially needed in the future is considered. Doors should be constructed of stainless steel or an alternative material and be able to withstand the decontamination procedure of the facility. The doors should be able to maintain the pressure gradient in the facility either using manual latches or automatic pneumatic seals to lock the doors in place [3].

2.5 PPM of primary containment

2.5.1 Cabinets

The highest level of protection afforded to the lab worker and surrounding environment is given by the BSCIII [4]. BSCIII use the combination of high negative pressure and HEPA filters to ensure aerosols are not released during work. This has been shown by Barbeito and Taylor, who demonstrated that even with the gloves removed from a class III cabinet it still afforded a high degree of protection, and contained all aerosols produced when running under normal operation [7].
The European standard (BS EN 12469) provides the testing regime and methods to ensure a BSCIII is operating to a safe level. Requirements stated within it are, for example, that the cabinet must operate at a negative pressure of less than -250 Pa and there must be a

volumetric inflow of air into the cabinet through a HEPA filter of >0.05 m3/sec-1 for every cubic meter of the cabinet's internal volume. This ensures that the flow rate through a glove port with the glove removed will be >0.7m/s, this equates to the inwards flow velocity produced by a class 1 biological safety cabinet [3].
Furthermore, class 3 cabinet when sealed and pressurised to 500 Pa must only lose ≤10% over a 30 minutes period, the UK HSE guidance for high containment laboratories states that for a cabinet line this initial pressure should be 150 to 250 Pa to reflect the difficulties achieving the high pressure stated for a single cabinet [3]. Testing of the Georgie State University's cabinet line, in the USA, involves demonstrating a leak rate of ≤2 x 10-5 cc/sec-1 of helium when pressurised to three inches of water column [8].
A maintenance and testing regime for the cabinets is not directly stated in guidance documents, but is noted it should be annually or more frequent depending on the use. It will therefore be the responsibility of the facility's operators to select a regime that is appropriate and be able to justify this to the country's regulators. For example at PHE Porton maintenance and testing is scheduled for every six months.

2.5.2 Positive pressure suits

Positive pressure suits used in high containment require regular inspection and maintenance to ensure it is operational. Visual inspection involves an overall examination then closer inspection of the welds and zips. This will be followed by a pressure hold test to determine if there are any undetected holes or defects. The British standard BS EN 464:1994 is currently suggested for pressure testing of suits used in high containment in the UK. The suit's exhaust valves are sealed and the suit is inflated to a pressure of 1750 Pa, where it is held for 10 minutes, then reduced to 1650 Pa for a further 6 minutes [3]. If the pressure is not maintained then the suit should not be used. Suits can be repaired with a waterproof repair kit and tested again. A replacement schedule can be implemented dependent on the procedures completed and the amount of use the suit receives.

2.6 PPM of HEPA filters

The HEPA filters are an integral component to the correct functioning of the cabinet line at BSL4 therefore it is imperative that they are tested and operating correctly. Generally, as manufacturing processes have become better, HEPA filters are constructed more robustly and fail less often. Failure usually occurs after the filter has been fitted, through insufficient even pressure across the gasket, causing damage to the gasket which can cause leakage [9]. In the UK in situ testing is completed using the British standard 6609:2007, and in the USA NSF/ANSI Standard 49—2007 is used. Both involve the testing of HEPA filters by generating an aerosol challenge upstream of the filter. The downstream face of the HEPA filter is then scanned using a detection probe, as well as the fixing gasket. HEPA filters installed in the facility should be tested regularly, this is specified as every 6 months within the UK by its regulators [3]. If HEPA filters are found to fail the testing they can be removed, sealed in an airtight bag and then disposed of by the appropriate method e.g. incineration or autoclave.

2.7 Requirement for external sensing

If the testing regime is followed correctly and the containment found to be performing to the specific standards used, then it will not be necessary to provide further monitoring to protect the environment,. unless there is a procedure that will cause loss of containment and then alternative arrangements can be put into place for this. Procedures can be assessed to see how much aerosol they generate by performing them with surrogate material or organisms and sampling the air in the close vicinity for the surrogate and to determine the particle size. This will allow for the estimation of the protection factor afforded by the containment and if special measures will need to be implemented to reduce and potential release.

2.8 Emergency procedures for leak in containment

If a leak is detected in the containment or facility an assessment will need to be completed as to the impact of this release. Risk assessment would have been performed prior to the commencement of work to identify what emergency steps should be taken for each scenario envisaged. Aspects such as the type of release (aerosol, liquid, solid material), likely amount of material released, specific environment likely to be contaminated (soil, watercourse) should be considered. Plans of action can be drawn up regarding things like the appropriate personal protective equipment (PPE) required for the workers and the most effective decontaminant to be used. These can then be inputted into the risk assessments and emergency procedures produced.

2.9 Waste handling

A laboratory produces waste from a number of different sources, which need to be treated to reduce any potential contamination to an appropriate level. Waste that will commonly be produced in a laboratory will be either in solid or liquid form. Depending on activities performed inside an ESCF, there is the risk of producing hazardous waste, e.g., as a result of destructive analyses, required for the life detection and biohazard assessment.
The amount of waste produced directly from the samples themselves will generally be small in mass or volume, but items that have been in contact with the sample will be categorised as contaminated and can either be decontaminated or disposed of.
Often it is easier, safer and more practicable to dispose of waste, whilst also helping to reduce cross contamination between samples. Solid waste can be placed into a waste bags and to which the exterior is decontaminated using liquid, then the waste bag is placed into another bag sealed and the external surface decontaminated using either liquid and/or gaseous decontaminant prior to its removal from containment. At BSL4 these items will be passed through an autoclave and the cycle use to sterilise the waste prior to its removal. Solid waste items can also be decontaminated prior to bagging if there are thought to be heavily contaminated. This is undertaken by placing the item in a container that is filled with the appropriate liquid decontaminant. They are kept in this liquid for a predetermined period when the waste container is then closed, double bagged and passed through the autoclave cycle [10].

Liquid waste is generally not directly allowed to leave the containment laboratory without decontamination. Effluent treatment plants will be used to treat any liquid waste that is produced from showering or lab sinks outside of primary containment.
If there is no autoclave available or the there is a requirement to remove unsterilised material, then a decontamination dunk tank can be used for a cabinet line laboratory. Waste material should be double bagged as previously stated and then submerged in the dunk tank for the required period of time, then removed and either incinerated or subjected to an autoclave cycle. Unsterilised material, such as sample material that has been subdivided into smaller fractions would be sealed in the appropriate container and then placed within another rigid container that would be submerged in the dunk tank, for the appropriate time, then removed and returned to the storage facility.
Other waste produce will be dependent on the equipment used. Some waste gases will be generated from technologies that heat samples to volatise them, any exhaust gas should be passed through a filter that will be able to stop particles of the determined size. It may be a requirement to use two filters in line from each other to ensure no particles are released.
Larger solid items that will be classed as waste could be items that require special decontamination regimes. HEPA filters used within a restricted return facility will be required to be fumigated prior to removal, then removed into a sealed bag for destruction autoclaving prior to being incinerated, which is the current practice in a high containment facility in the UK. However, future research may modify these practices and determine a more practicable course of action.
Decontamination processes are validated using either chemical (CI) or biological indicators (BI), or physical technology. Biological indicators are hardy organisms to that process that will be applied to a representative substrate and exposed to the process. The organism's population placed onto the substrate can be varied, i.e. to represent more stringent challenges more organisms can be used. The biological indicators can then be subjected to the decontamination process, on completion BIs can be processed to see if there are any viable organisms. The organism can be changed depending on the decontamination process. For example for formaldehyde fumigation spores of Bacillus atrophaeus are used, but for gaseous hydrogen peroxide applications spores of Geobacillus stearothermophilus are used [11]. G. stearothermophilus spores are also used for autoclave cycle validation. Chemical indicators are created by adding a certain chemical that will change colour after exposure to the decontamination method at a certain concentration or for a time period. These are more of an aid rather than validation because they can still change, or start to change colour even if the peak decontaminant concentration has not be reached. Physical methods of validation, such as thermocouples or direct measurement of the parameters of the process can be used to determine an effective decontamination process. For instance the direct measurement of temperature and pressure within an autoclave allows for an immediate identification if the procedure has reached the required parameters. The cycle can then be aborted and the issue identified and rectified.

2.9.1 Framework Legislation

In general terms, the European approach to waste management is based on the "waste hierarchy" which sets the following priority order when shaping waste policy and managing waste at the operational level: prevention, (preparing for) reuse, recycling, recovery and, as the least preferred option, disposal (which includes landfilling and incineration without energy recovery).
In line with this, the 7th Environment Action Programme sets the following priority objectives for waste policy in the EU:

- to reduce the amount of waste generated,
- to maximize recycling and re-use,
- to limit incineration to non-recyclable materials,
- to phase out landfilling to non-recyclable and non-recoverable waste,
- to ensure full implementation of the waste policy targets in all Member States.

Hazardous wastes may pose a risk to the environment and human health and require a stricter control regime. This is laid down in particular in Articles 17 to 20 of Directive 2008/98/EC. It provides additional labelling, record keeping, monitoring and control obligations from the waste producer to the final disposal or recovery. In addition, mixing of hazardous substances is banned in order to prevent risks for the environment and human health. Moreover, the permit exemptions that may be granted to installations dealing with hazardous wastes are more restrictive than for installations dealing with other wastes.
The classification into hazardous and non hazardous waste is based on the system for the classification and labelling of dangerous substances and preparations, which ensures the application of similar principles over their whole life cycle. The properties which render waste hazardous are laid down in Annex III of Directive 2008/98/EC and are further specified by the Decision 2000/532/EC establishing a List of Wastes as last amended by Decision 2001/573/EC.
It has to be remarked that, despite the similar methodological approach to both chemical and biological waste and the effort to highlight common issues, there are inevitable differences (technical, legal, etc.) between the two areas.

2.9.2 Waste management

The hazardous waste management deals with:

- **Storage**: Temporarily holding hazardous wastes until they are treated or disposed. Hazardous waste is commonly stored prior to treatment or disposal, and must be stored in containers, tanks, and containment buildings.
- **Treatment**: Using various processes, such as incineration or oxidation, to alter the character or composition of hazardous wastes. Generally speaking, some treatment processes enable waste to be recovered and reused in manufacturing settings, while other treatment processes dramatically reduce the amount of hazardous waste.
- **Disposal**: Permanently containing hazardous wastes. The most common type of disposal facility is a landfill, where hazardous wastes are disposed of in carefully constructed units designed to protect groundwater and surface water resources.

2.9.3 Waste facility requirements

Within the ESCF, hazardous waste should be collected, treated in order to reduce their amount and dangerousness, stored in a safe area and then disposed.
The waste should be collected as soon as possible, once it is produced (i.e., because of a sample analysis) and immediately stored in containers and tanks:

- Hazardous waste containers are portable devices in which a hazardous waste is stored, transported, treated, or otherwise handled.
- Tanks are stationary devices used to store or treat hazardous waste. Tanks are constructed of a wide variety of materials including steel, plastic, fiberglass, and concrete.

Hazardous chemical and biological waste should then be treated. A possibility is the use of incinerators, enclosed devices that use controlled flame combustion for the thermal treatment of hazardous waste. When performed properly, this process destroys toxic organic constituents in hazardous waste and reduces the volume of waste that needs to be disposed. The use of incinerators or similar devices inside or in the nearby of the ESCF is not mandatory. This is the reason why the ESCF should have a containment area: a completely enclosed, self-supporting structures (i.e., they have four walls, a roof, and a floor) used to store the hazardous waste. The containment area should be accessible from outside the SRF/SCF units of the facility, to pick up the waste for the following disposal outside of the ESCF or in another part of the ESCF itself. The containment area may contain tanks, if needed.

2.10 Security

A sample receiving facility will require a number of different approaches to maintain the security of the facility, staff, samples and the information generated from the testing regime. Physical security elements are required in order to prevent unauthorised access and protect the samples from removal for non-official purposes [4]. They can be site specific and depending on the location of the ESRF, the site might already have a number of these security elements in place i.e. if the ESRF is located at an existing facility. Security criteria will be stipulated by the facility's host control. For instance in the USA the Federal Select Agent Program which oversees the use and possession of selected biological agents and toxins that pose a severe threat to human health and the environment (https://www.selectagents.gov/). In the UK the Anti-terrorism, Crime and Security Act 2001, Schedule 5, details the pathogens and toxins that are covered to enhance the security of the most dangerous material (http://www.legislation.gov.uk/ukpga/2001/24/schedule/5). Part of these requirements is to ensure the storage of materials detailed within them are as secure as practicable. It would be envisaged that until it is proven that no lifeforms are contained within restricted return samples then they will be covered by these acts or ones pertaining to other countries, depending on the location of the SRF. It would be a requirement of the design of the facility to contact the national security office of that country to discuss security arrangements that will be needed for the facility.

There are a number of different layers to the security of the facility starting with security element designed to limit the access to the facility to those who are authorised and trained to work in it. For staff members this may be through background security checks/vetting. This can be a requirement by the host country's government for workers handling highly pathogenic agents or classified information. This will also apply for visiting or foreign workers to the facility.

Physical security around the site should include at least robust security perimeter fencing, 24 hour manned security and a number of different layers of access control to the laboratories [3] (https://www.gov.uk/guidance/secure-hazardous-materials-to-help-prevent-terrorism). Internal security will comprise of sectors that are access controlled to only allow those staff with the correct level of clearance through them. The security can be maintained using combination locks, security key cards or other biometric controls (e.g. finger print scan, retina scan or vein matching). Further security controls should be implemented on storage of access doors to areas where samples can be contacted. Security access will also allow for the recording of movements of individuals and locating them during emergencies.

Security arrangements will need to be considered for the information generated from the experiments conducted within the facility. The level of security will be identified during the design and planning phase of the project. Security arrangements could be secure servers for the storage of electronic information and restricted access to equipment.

2.10.1 Risks and security domains

According to "Prudent practices in the Laboratory", risks to laboratory security include:

- Theft or diversion of chemicals, biologicals, and radioactive or proprietary materials (such materials could be stolen from the laboratory, diverted or intercepted in transit

between supplier and laboratory, at a loading dock, or at a stockroom, and then sold or used, directly or as precursors, in weapons or manufacture of illicit substances),
- Theft or diversion of mission-critical or high-value equipment,
- Threats from activist groups,
- Intentional release of, or exposure to, hazardous materials,
- Sabotage or vandalism of chemicals or high-value equipment,
- Loss or release of sensitive information,
- Rogue work or unauthorized laboratory experimentation,
- Terrorism.

The type and of the security systems applied inside the ESCF not only depend on the regulatory requirements but also on the mission of the facility. A good security system should, among other things, increase overall safety for laboratory personnel and the public, improve emergency preparedness by assisting with preplanning, and lower the organization's liability. There are four integrated domains to consider when improving security of a facility:
- **Architectural**: doors, walls, fences, locks, barriers, controlled roof access, and cables and locks on equipment,
- **Electronic**: access control systems, alarm systems, password protection procedures, and video surveillance systems,
- **Operational**: sign-in sheets or logs, control of keys and access cards, authorization procedures, background checks, and security guards,
- **Information**: passwords, backup systems, shredding of sensitive information.

Security systems should help to:
- detect a security breach, or a potential security breach, including intrusion or theft,
- delay criminal activity by imposing multiple layered barriers of increasing stringency or "hardening" in the form of personnel and access controls,
- respond to a security breach or an attempt to breach security.

2.10.2 Infrastructural security

Physical and electronic security contribute to define the infrastructural security, which begins at the perimeter of the building and becomes increasingly more stringent moving toward the interior area, where sensitive material, equipment, or technology reside.
This requires that the ECSF layout be defined as more concentric as possible as shown in Figure 10.

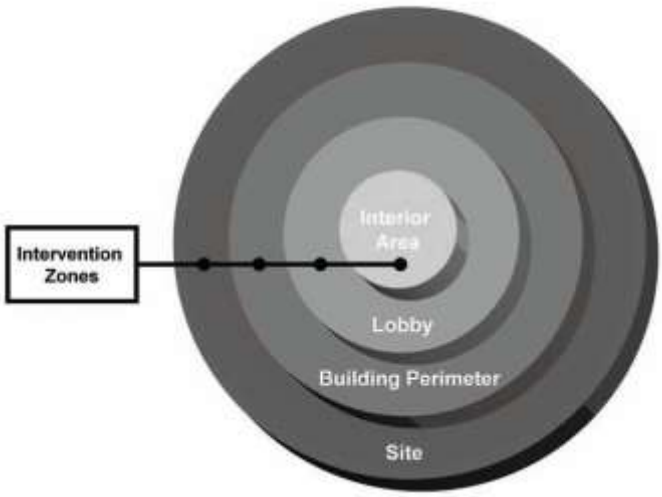

Figure 10. Layout defined as concentric.

Using the infrastructure units shown in Paragraph 1.1 of this Deliverable, the concentric infrastructural security should be redefined as in Figure 11.

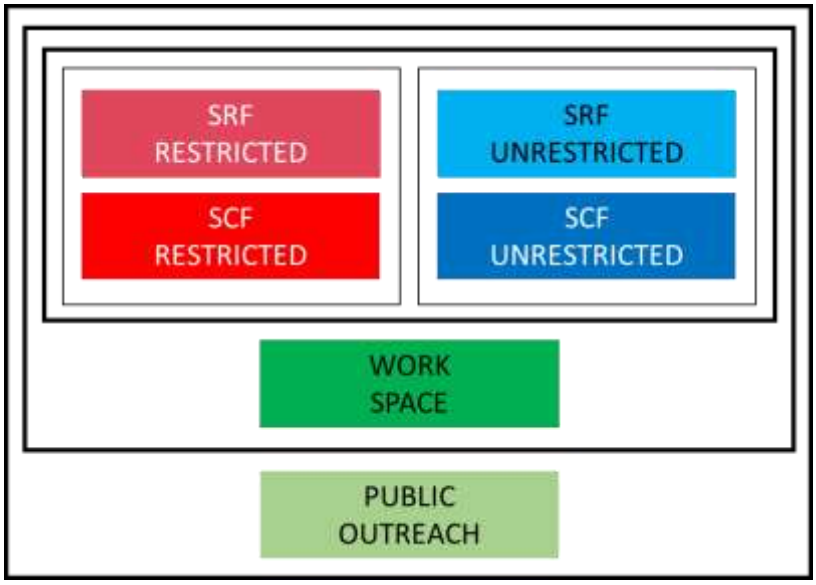

Figure 11. ECSF layout defined as concentric.

Elements of infrastructural security include:

- Door locks,
- Video surveillance,
- Glass-break alarms for windows and doors,
- Intrusion alarms,

- Hardware to prevent tampering with window and/or door locks,
- Lighting of areas where people may enter a secure area,
- Bushes and other barriers to reduce visibility of sensitive areas from outside the building,
- Locks on roof access doors,
- Walls that extend from the floor to the structural ceiling,
- Tamper-resistant door jambs,
- Blinds on windows,
- Locks and cables on equipment to prevent easy removal,
- Badges or other forms of identification,
- Sign-in logs.

2.10.3 Operational security

Operational security is responsible for the people within the laboratory. A security system is only as strong as the individuals who support it, and thus, among the goals of an operational security system are to increase awareness of security risks and protocols, to provide authorization for people who need access to a given area or material, and to provide security training.
Elements of operational security include:

- Screening full- and part-time personnel before providing access to sensitive materials or information,
- Providing ID badges,
- Working to increase the situational awareness of laboratory personnel (e.g., traceability of the personnel inside the facility, identifying suspicious activity),
- Encouraging the reporting of suspicious behavior, theft, or vandalism,
- Restricting off-hour access to laboratories,
- Providing entry logs at building and laboratory access points,
- Inspecting and inventorying materials removed from the laboratory.

2.10.4 Information security

Information is critical as security of equipment and materials. Loss of data and computer systems from sabotage, viruses, or other means can be devastating for a laboratory. The most important countermeasure to preserve safely the information is to develop and institute a plan for backing up data on a regular basis with backup media off-site, in fire-safe storage, at a central (e.g., the institution's information technology facility) or a mirror facility.
If the laboratory produces private, sensitive, or proprietary data,

- Provide training to those with access to this information, stressing the importance of confidentiality. Review any procedures for releasing such information outside the laboratory or group.
- Consider a written and signed confidentiality agreement for those with access to such information.

- Keep passwords confidential, do not store or write them in an obvious place.
- Change passwords routinely.
- Safeguard keys, access cards, or other physical security tools.
- Before discarding materials that contain sensitive information, render them unusable.
- Report any known or suspected breaches in security immediately.
- Establish policies and procedures for the storage of proprietary information on hard drives or portable storage media and for the removal of proprietary information from the laboratory or secure area.

2.10.5 Biological security

In a facility, materials may have both a bona fide use in scientific research, but also can be used for criminal or terrorist activities. Certain biological agents, (i.e., viruses, bacteria, fungi, and their genetic elements) may have a potential for use by terrorists to harm human health. Furthermore, biological materials can replicate, thus, theft of even small amounts is significant.
This applies also to the ESRF, which, in case of samples showing the possibility of extinct or extant life, should deal with biological agents. Particularly given the uncertainty on the nature of any biological material brought back to Earth
Following the guidelines proposed at the state of art (i.e., "Biosafety in Microbiology and Biomedical Laboratories", 2007), the security of biological materials should be defined following the regulators statement, using a risk assessment tool.

2.10.6 Security management

Any security plan should identify a person or group responsible for the overall plan. The person or group managing the program should have at least basic security knowledge, understand the risks and vulnerabilities, and should be provided sufficient resources, responsibility, and authority.

2.10.7 Training

Security should be an integral part of the laboratory safety program. Ensure all personnel are trained in security issues, in addition to safety issues. Although safety and security are two different things, there are many overlaps between measures used to increase security and those used to increase safety, including

- Minimizing the use of hazardous and precursor chemicals, which reduces health, safety, and potential security risks,
- Minimizing the supply of hazardous materials on-site,
- Restricting access to only those who need to use the material and understand the hazards from both a chemical standpoint and a security standpoint,
- Knowing what to do in an emergency or security breach, and how to recognize threats.

Ensure that all personnel understand the security measures in place and how to use them. No matter how complex a system may be, the weakest link tends to be personnel.

2.11 Communications

There should always be a method of communicating directly to the laboratory or laboratory suite. This is usually through a hands free telephone which removes the potential for contamination of the worker's face or person. Person to person communication within a cabinet line laboratory can be achieved easily but a different system will be required when the operators are working in positive pressure suits as these can be noisy and difficult to communicate in, even when two workers are stood together. In these instances radio communication systems are employed with push to talk microphones. This allows easy communication to others in the laboratory or outside of the laboratory. One security issue with radio usage is that people external to the facility could intercept the communications within it. This can be overcome by using a secure frequency and encrypted radios.

2.11.1 Communication net

An ESCF communication plan is mandatory: laboratory personnel should be aware of the plan and should know what to expect and what is expected of them.
Following the concentric infrastructural security approach, the more sensible are the activities performed inside the facility units (i.e. the SRF and SCF), and the more redundant and self-powered the communication devices should be. This applies to both the internal communications systems (from e to each facility unit) and the external ones (from the facility to the outside and vice-versa).
The ESCF should be then considered as a net, where each unit is a node.
If no unexpected incident occurs inside the facility, the communication net is shown in Figure 12.
It has to be noticed that, for graphical reasons, not all the connections are showed in the Figure: communications is possible "across" an unit, when it has two or more connections (i.e., SRF restricted unit can directly communicate with the work space unit, even if there isn't a direct connection).
In this case the work space unit acts as an information collector, connected to all the other ESCF units and to the outside.

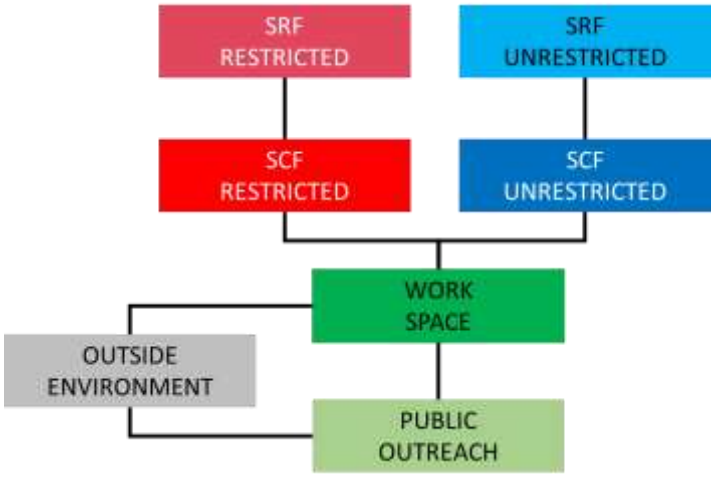

Figure 12. ECSF communication net

Otherwise if an emergency affects the facility (i.e., some regular means of communication may be compromised: telephones may not work, a power loss may affect access to computers, etc.), all the units are connected to each other and to the outside. Figure 13 shows the communication net in case of emergency.

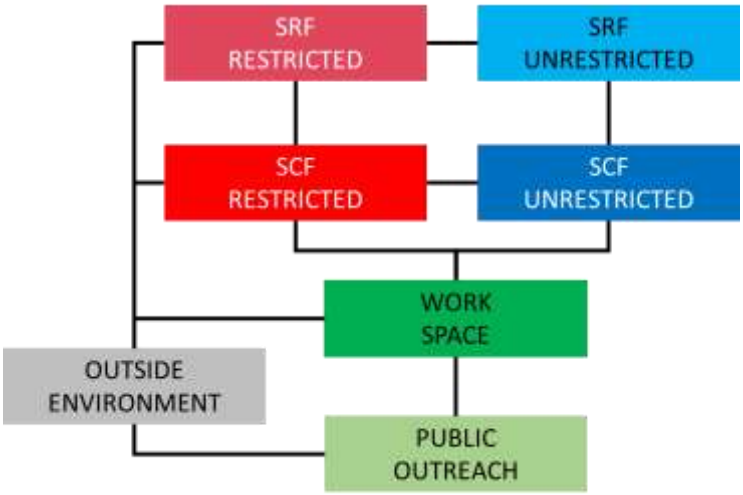

Figure 13. ECSF emergency communication net

This is particularly important in case of security issues when, depending on the circumstances, some connections may have a failure.

2.11.2 Redundant systems

Redundant systems are mandatory in case of unexpected incidents when an emergency affects the ESCF or the surrounding environment. In this cases telephone systems may quickly become overloaded, and local or institutional police, security, or public safety officials

may be bombarded with calls. Text messaging, e-mail, and the internet can be the primary redundant means of communication. Self-powered devices are also mandatory in case of extremely serious incidents.

2.11.3 Communication to media

If an incident has caught the attention of the media, the institution's spokesperson must be involved in any conversations with reporters. Media inquiries should go through the person or group that is used to working with the media, because it is very easy for facts or issues to be misconstrued or presented in an inflammatory manner. All involved should be instructed to forward calls and interviewers to the media relations group. When an incident command system has been instituted, a press officer will be appointed. All inquiries and statements go through this individual or group.

3 Processing requirements in terms of number of operations and amount of flow between the different facility elements

3.1 Planetary Protection units

Once the units are defined, so as some possible different Storage and Curation Facility layouts, we can focus on the units where Planetary Protection activities are performed: SRF Restricted and SCF Restricted.
Both the units are clean and high containment environment. For both units, a list of the main areas (or subunits) is shown in the Figure 14.

SRF RESTRICTED
Receiving Area
Cleaning Area
Opening Area

SCF RESTRICTED
Opening Area (last layer)
BAP Area
Animal Tissue Testing
Curation Area
Preparation Area
Sterilization Area
Storage Area (Samples)
Storage Area (Consumables)
Storage Area (Hardware & Coupons)

Figure 14. SRF Restricted and SCF Restricted

3.2 Workflow of Planetary Protection activities

According to D2.1, the Life Detection/Biohazard Assessment procedures follow the sequence displayed in Figure 15.

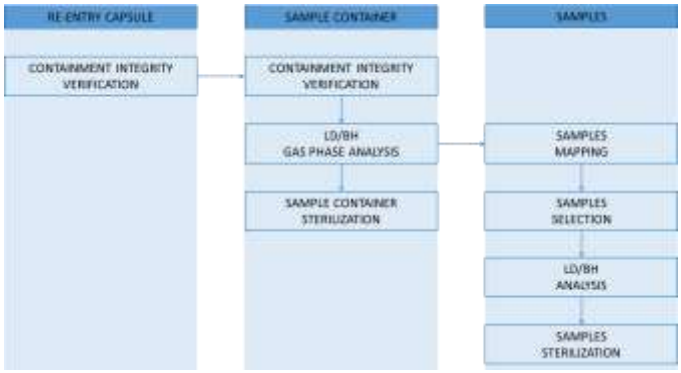

Figure 15. Sequence for Life Detection/Biohazard Assessment procedures.

In order to distribute all these previous procedures in the units presented in the previous paragraphs, we can follow the swim-lane flow chart presented in Figure 16, where each curation activities is assigned to a specific unit.

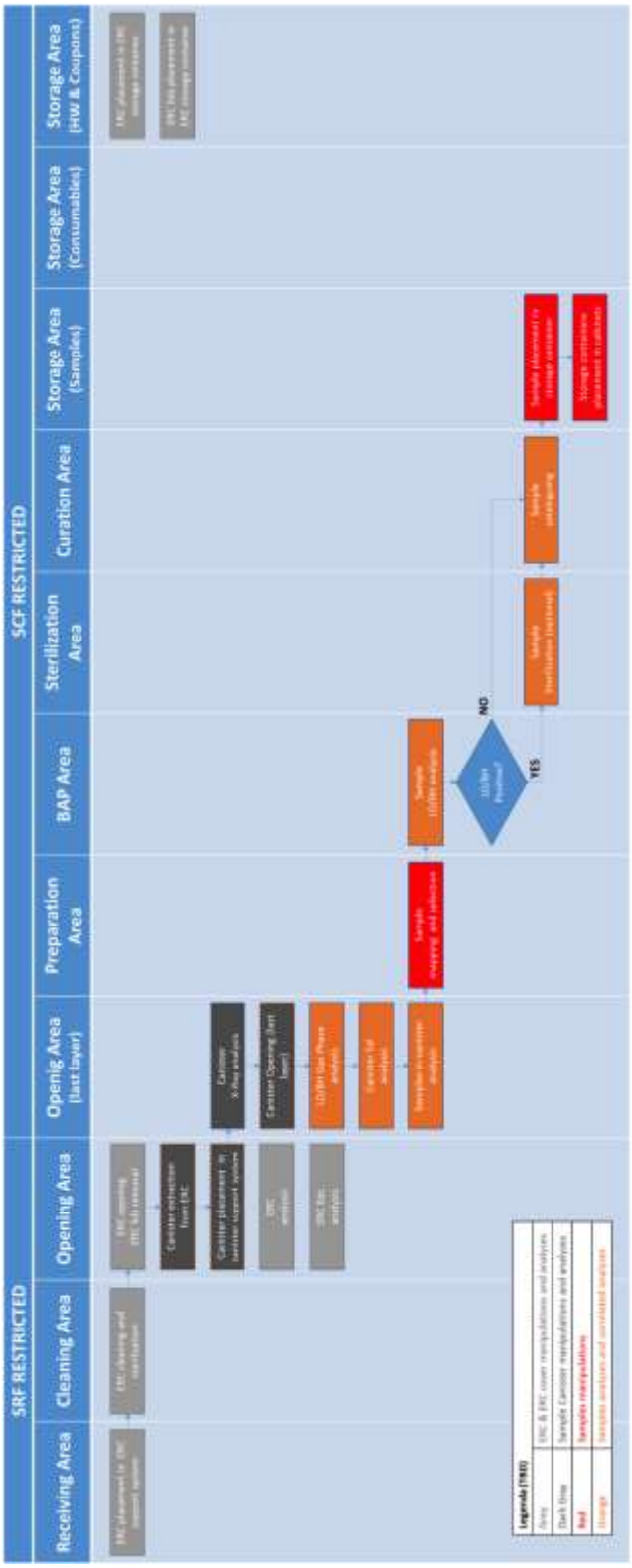

Figure 16. Swim-lane flow chart shows areas and procedures for Curation Facility

All the mentioned activities require a planetary protection assessment. It is clear that the major issues arises from the activities performed on the samples. It requires an estimation of the number of operations and the amount of flows needed on them.

3.3 Workflow of samples

First of all we have to consider the total amount of samples available (TS) for the operations. The first step requires that the amount TS be devised 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.* (2002) proposed a 10% as a rough estimate of a reasonable amount of sample to be used. De Vincenzi and Bagby (1981) used the same figure. Furthermore, sample-sizing calculations were assumed by MEPAG E2E-iSAG (2011) for planetary protection aspects and the mass of 1.5 g of each sample was 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 = SSC

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

Figure 17 resume the entire flow.

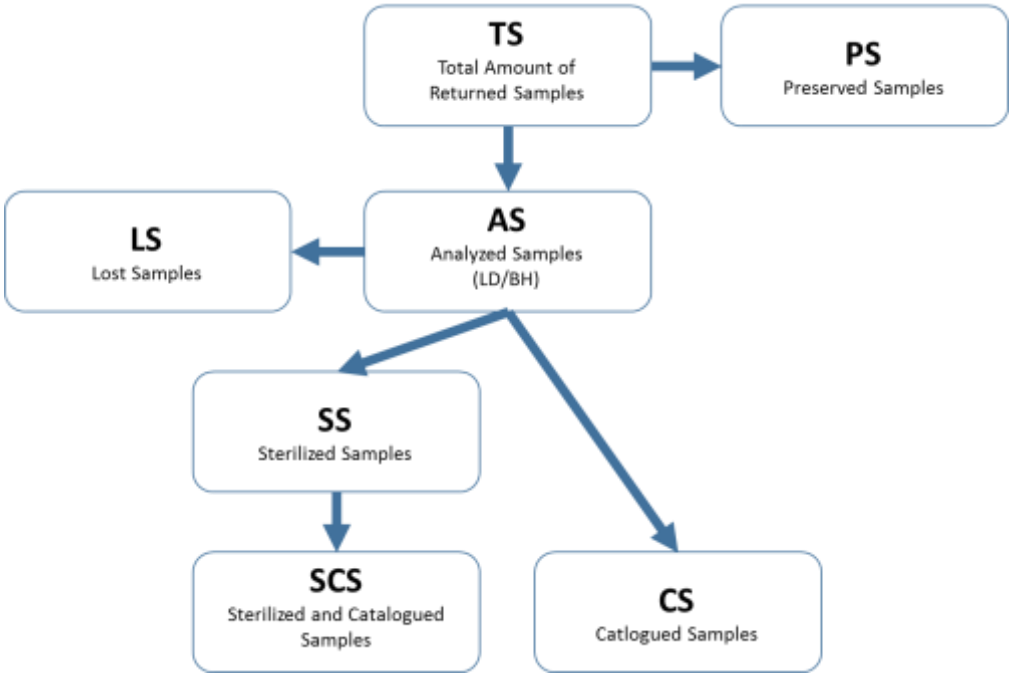

Figure 17. Workflow of samples inside Curation Facility

3.4 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 Figure 18, where the amount of AS versus the TS is shown.

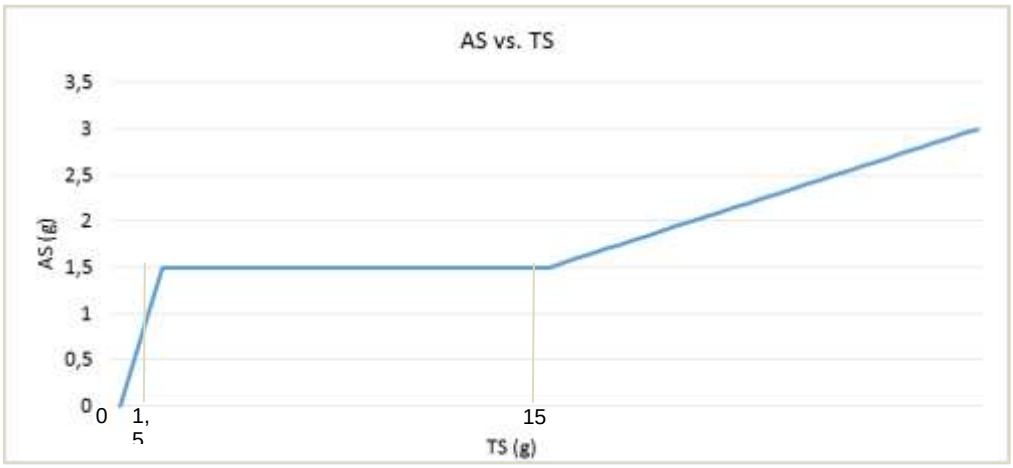

Figure 18. Amount of sample required for LD and BH (AS) according to the total amount returned by space mission (TS).

The AS has to be subjected to LD/BH assessment, using the instruments shown in Figure 18 (from deliverable D2.1). Figure 19 show a list of instrument, associated to biosignatures and ranked by a technique importance rate.
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, it 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, using the data shown in Figure 19, 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.

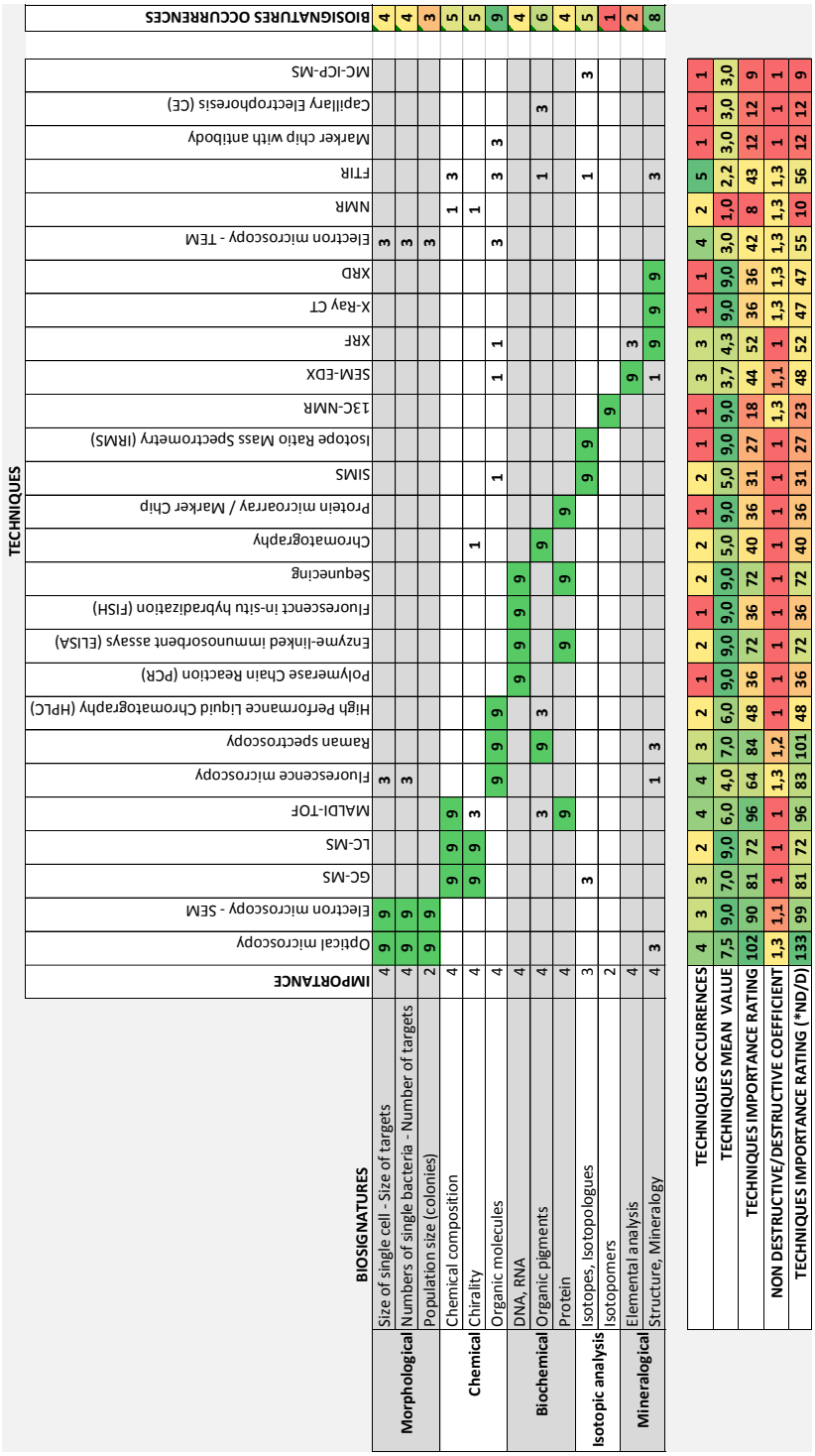

Figure 19. Biosignatures vs. Techniques

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