D3.3 ADVANCED DESIGN AND TECHNOLOGY IDENTIFICATION

WP3 – FACILITIES AND INFRASTRUCTURE

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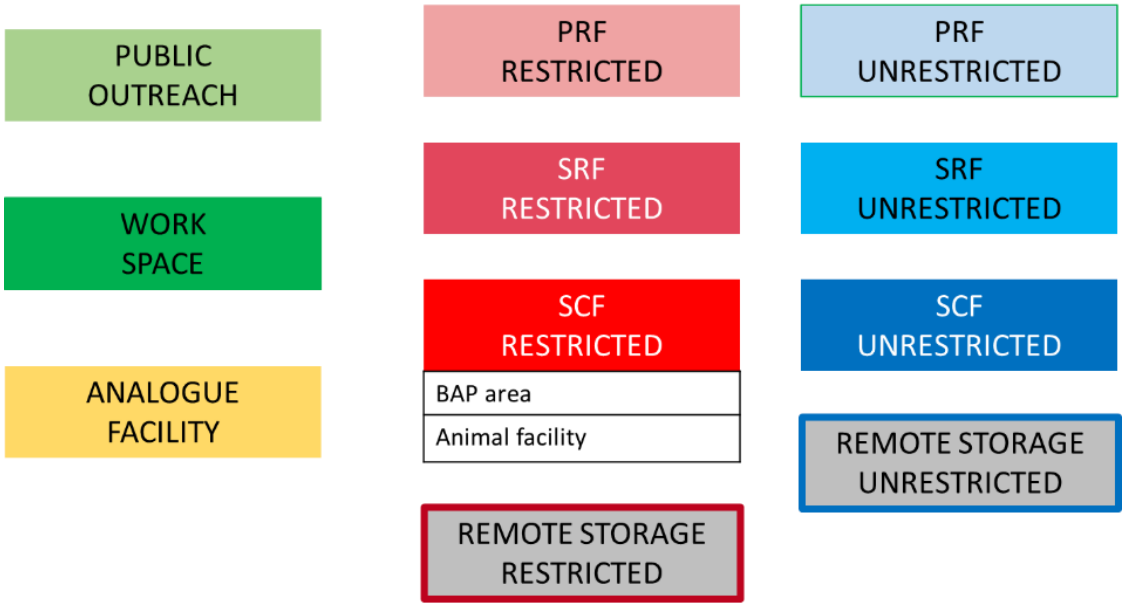
I. Introduction

This report presents an Advanced Conceptual Design of the European Sample Curation Facility (ESCF). It has been produced as a continuation of the previous deliverables (D3.1 and D3.2), by adding knowledge acquired through visit of facilities, meetings with experts and research on specific topics.

Considering the deliverable D2.5 of Work Package 2 (WP2), we do not elaborate on infrastructure, curation and storage issues for restricted samples. The next deliverable (D3.4) will take inputs from D3.3 and D2.5 to offer a full and comprehensive final design.

II. Parts of the facility

As presented in our previous deliverables, we recommend a modular approach for the ESCF, with independent units built over time when necessary. Below is a reminder of the modules and independent sub-modules (for SCF Restricted). For more details, see D3.1. Acronyms are defined at the end of the document.



Two tables are presented below showing the areas of each scientific module, with coefficients applied for parameters such as Cleanliness level, Frequency of use, etc. for unrestricted and restricted missions. This trade-off was used to develop the flowcharts presented in the next section.

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Parameters are rated as follow:

- Frequency of use: 1 = every day; 3 = every week; 9 = rarely
- Cleanliness level: 1 = ambient; 2 = medium (ISO 7-8); 3 = clean (ISO 5-6); 4 = extra-clean (ISO 4)
- Containment level: 1 = none; 2 = medium (BSL-2/BSL-3); 3 = high (BSL-4)
- Humans versus Robots: 1 = Humans only; 2 = Robots only; 3 = both possible

	Areas	Frequency of use	Cleanliness level	Humans vs. Robots	Air shower to enter	Changing room
SRF						
#01	Receiving area / High bay	9	1	3		Y
#02	Opening area	9	3	3	Y	Y
#03	Cleaning area	9	3	3	Y	Y
SCF						
#01	Opening area	9	4	3	Y	Y
	CURATION					
#02	Preliminary Examination area	1	4	3	Y	Y
#03	Sample preparation	3	3	3	Y	Y
#04	Storage area (Samples)	3	4	3	Y	Y
#05	Storage rooms (consumables)	1	3	3	Y	Y
#06	Storage area (HW & Coupons)	9	4	3	Y	Y
#07	Cleaning tools area	1	3	3	Y	Y
#08	Storage area (readmitted samples)	9	3	3	Y	Y
#09	Monitor room	1	1	1	N	N
#10	Distribution	3	3	3	Y	Y
	MACHINING					
#11	Air handling HVAC floor	9	1	1	N	N
#12	Machining room(s)	9	1	1	N	N
#13	Water supply area	9	1	1	N	N
#14	Effluent treatment area	9	1	1	N	N

Table 1. Unrestricted part of the ESCF with trade-off. SRF and SCF are independent modules. Areas are dependent.

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	Areas	Frequency of use	Cleanliness level	Containment level	Humans vs. Robots	Air shower (entry)	Changing room	Decontam. shower (exit)
SRF								
#01	Receiving area	9	1	2	3		Y	N
#02	Opening area	9	3	2	3	Y	Y	N
#03	Cleaning rooms	9	3	2	3	Y	Y	N
SCF								
#01	Opening area	9	4	3	3	Y	Y	Y
	CURATION							
#02	Preliminary Examination area	1	4	3	3	Y	Y	Y
#03	Sample preparation area	3	3	3	3	Y	Y	Y
#04	Cleaning tools area	1	4	3	3	Y	Y	Y
#05	Storage area (Samples)	3	3	3	3	Y	Y	Y
#06	Storage rooms (consumables)	1	4	1	3	Y	Y	N
#07	Storage area (HW & Coupons)	9	3	2	3	Y	Y	Y
#08	Monitor room	1	1	1	1	N	N	N
#10	Sterilisation area	9	3	3	3	Y	Y	Y
	MACHINING							
#11	Air handling HVAC floor	9	1	1	1	N	N	N
#12	Machining room(s)	9	1	1	1	N	N	N
#13	Water supply area	9	1	2	1	N	N	N
#14	Effluent treatment area	9	1	2	1	N	Y	N
Biohazard Assessment Protocol (BAP)								
#01	BAP area	1	3	3	3	N	Y	Y
#02	Storage Area (Samples)	3	3	3	3	N	Y	Y
#03	Storage rooms (consumables)	1	3	1	3	N	Y	Y
#04	Cleaning tools area	1	3	3	3	N	Y	Y
#05	Monitor room	1	1	1	1	N	N	N
#07	Air handling HVAC floor	9	1	1	1	N	N	N
#08	Machining room(s)	9	1	1	1	N	N	N
#09	Water supply area	9	1	2	1	N	N	N
#10	Effluent treatment area	9	1	2	1	N	Y	N
Animal facility		1	2	3	1	N	Y	Y

Table 2. Restricted part of the ESCF with trade-off. SRF and SCF are independent modules, BAP and Animal Facility are independent sub-modules of SCF. Areas are dependent.

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III. Workflows

Workflows are presented for operations and workers in Figures 1 and 2, for unrestricted SRF and SCF, based on our current understanding. They do not reflect the evolution over time. These workflows should be taken as preliminary versions, and will likely be updated in future deliverables.

1. Operations

Figure 1. See on page 8.

This workflow aims at showing the operations to be conducted in the facility, with the specific area in which they should be conducted. Cleanliness levels have been indicated, so we can reflect on the physical connections between areas, the flow of workers (Figure 2) and samples.

We have differentiated operations where the sample can be left inside the container (yellow boxes) and the operations where the sample will need to be taken out of the container (orange boxes).

2. Workers

Figure 2. See on page 9.

This workflow aims at showing which physical links should exist between different areas, to allow a smooth path of the human workers. It might be modified, depending on the importance given to robotics in the ESCF.

Maintenance staff comprises three categories of workers, with different security clearance, and different frequencies of frequentation:

- Cleanroom technicians, mostly for cleanroom instruments, on a daily basis.
- Facility engineers, for technical areas (power supply, air filtration…), on a daily basis.
- External companies, in both technical and curation areas, for periodic service and maintenance.

Cleaning staff will not access clean room and other controlled areas. Cleaning of sensitive areas (such as clean rooms, containment rooms, etc.) will be performed by the appropriate Curators/Technicians.

Security staff should be allowed in unclean/unrestricted areas, and through viewing corridors.

Workflows have been developed mainly for Curators and Technicians as they will access the facility on a daily basis and so mapping of their activities is of most importance to providing information to decide on adjacencies for the facility.

We suggest that some rooms are visible through viewing corridors. This can be adapted to the requirements of the facility.

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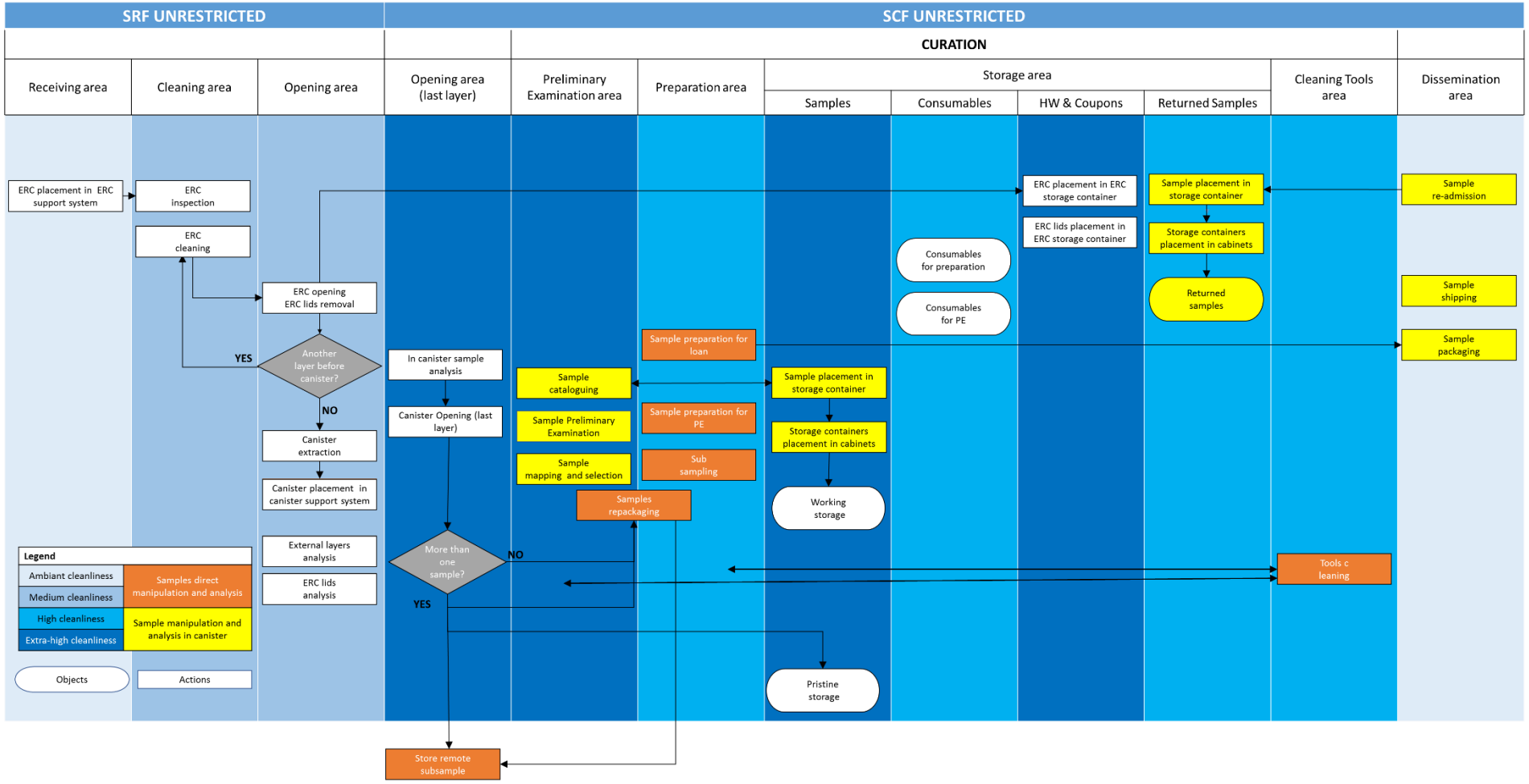

Figure 1. Flow of operations for unrestricted science areas.

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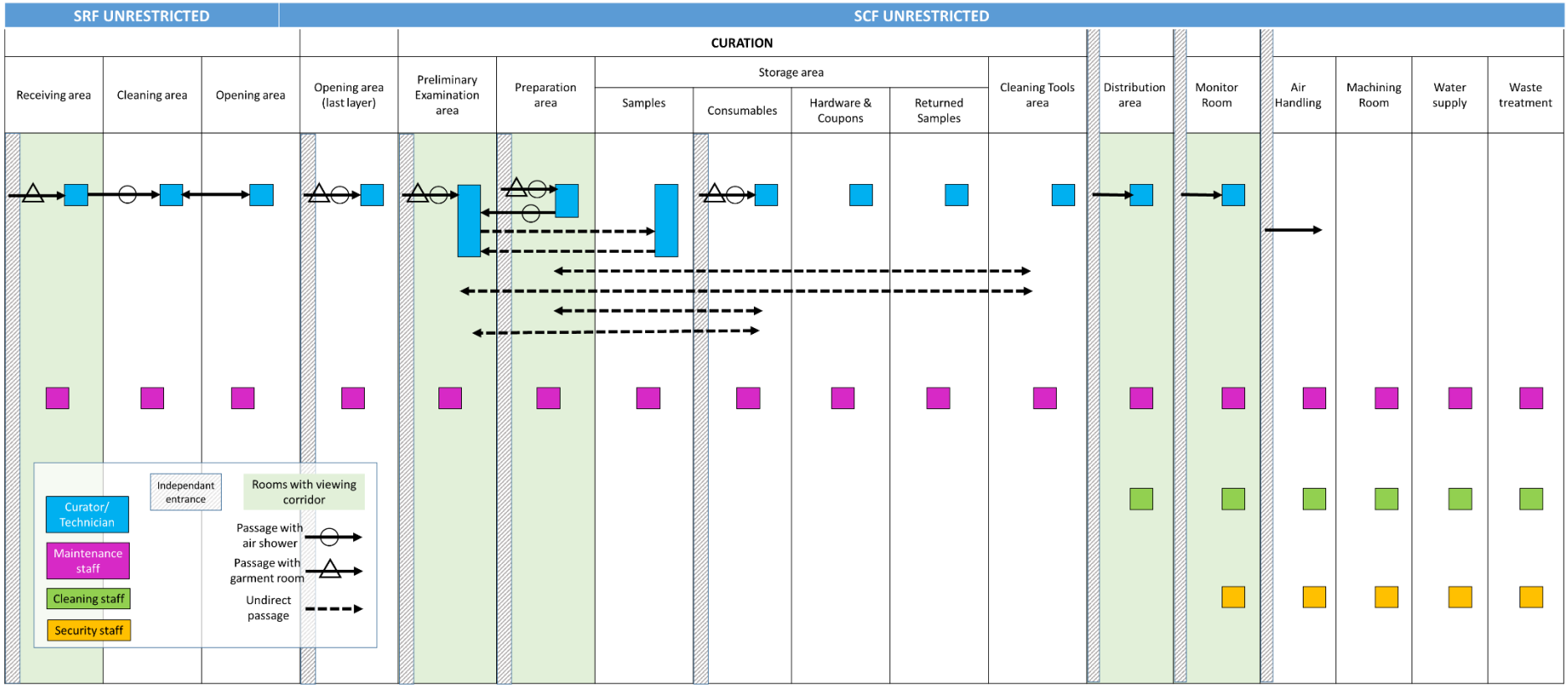

Figure 2. Flow of workers.

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IV. Curation

This section describes and discusses the main types of curation work within the facility with a specific focus on the personnel and technology (equipment) needs and requirements. In order to be able to properly plan for the curation of these precious samples, it is imperative that the curation activities, processes and equipments are considered at the earliest possible stage of mission planning.

The role of curation in the facility is two-fold, in the first instance the curation activity begins on receipt of samples and the preliminary examination (PE) phase. Once preliminary examination is completed then curation *sensu stricto* begins i.e. where the samples are carefully stored, handled and managed as a valuable scientific resource for generations of researchers to study. Whilst there are similarities in many of the curatorial processes during both phases of activity, there are some differences in personnel and equipment resources that will be required.

During sample receipt and PE, there is a close interplay between the needs of the scientific investigations and those of the curation and management of the samples. For Mars samples there is the added issue of the critical role of planetary protection and safety. Given that it may be the case that the exact nature of the samples is uncertain e.g. if a core sample has remained intact or whether frozen samples have remained frozen, it is critical that a high-degree of flexibility in terms of both technology and personnel training/experience is factored in. An increased number of personnel will be required initially. These personnel may be made up of a combination of permanent staff and visiting or contractual staff. Experience from previous sample return missions indicates that there is intense pressure to obtain results quickly, and fatigue within science teams and technical staff is likely to be an issue. One member of the Lunar Sample Preliminary Examination Team (LSPET) described being in a "daze of exhaustion" after three weeks of analysing Apollo 11 samples (Taylor, 1994). In a curation setting, especially for Mars samples, fatigue could further lead to breaches in protocol that could undermine both scientific and public confidence. Accordingly, considerable attention should be paid to developing plans for mitigating fatigue issues – for example, by having extensive training and a program of rotating staff, especially during the first few weeks to months after sample return.

Sample cataloguing will begin as soon as the samples are removed from their containers. This cataloguing will serve as the permanent record for each sample and will include many types of information and data. The cataloguing system will need to follow a standard and methodical approach. Such methodologies are currently used in many international institutions such as museums and national laboratories and many electronic cataloguing and databasing solutions are available. Curatorial procedures/workflows should be incorporated into the cataloguing system. As with any IT system, considerations must be made as to system security, both in internal use and for circumstances where external users can access the database e.g. external scientists providing or searching for information. Sample cataloguing will be a regular and constant part of the curatorial tasks during preliminary examination and well into the future.

Sample handling and manipulation systems will have to be capable of handling samples of different shapes and sizes and personnel operating those systems will have to be trained to deal with different types of material. The use of analogue materials will be very useful in this context as technologies/equipment can be tested prior to being confirmed for use in the facility and personnel can also be trained using these materials. During sample handling and manipulation there will be

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close collaboration between the curation personnel who is carrying out these operations and the scientists who are participating in the Preliminary Examination. Sample handling and preparation will be a regular occurrence (happening on a daily basis) during the Preliminary Examination phase and shortly after as the samples will be of great interest to the scientific community. However, over time it is anticipated that the requests for new samples will gradually diminish with the reuse of samples that have already been prepared for earlier work e.g. polished sections. It will be important that the skills honed by personnel during the early stages are maintained and also passed onto new personnel as necessary. This could be achieved through regular training on analogue samples.

There will be a high need for sample preparation equipment, for example saws, splitters etc. This preparation equipment will be needed to prepare samples for both internal and external scientific investigations. Again a degree of flexibility will be required given the possibility for a wide variety of different sample shapes and sizes. The selection of samples and the preparation of samples will be carried out in close collaboration with scientific investigators. For some scientific investigations, samples will have to be prepared in a certain way e.g. to a minimum or maximum size, with a flat surface etc. Given that the types of scientific instrumentation available within the facility and outside the facility will be known in advance, it should be possible to ensure that the required sample preparation equipment is available and that personnel is experienced in using it. Again, the use of analogue materials to test and verify the sample preparation equipment and techniques prior to installation in the ESCF and to train staff, will be highly beneficial. A major concern is sample cross-contamination, especially for samples where multiple samples have been collected from different environments and/or for samples collected from Mars (indeed given current exploration plans it is likely that when Mars Sample Return occurs, samples will be collected from different geological environments on Mars). To avoid sample cross-contamination, all curation equipment e.g. sample handling and preparation tools should be able to be thoroughly cleaned between operations on different samples. For Mars samples, this cleaning would also include sterilisation to comply with biohazard safety requirements. In the situation where equipment cannot be cleaned to the required levels, then it would be necessary to replace this equipment with new equipment. Depending on the type of equipment it may be possible that only the part(s) in direct contact with the samples would require replacement rather than the entire equipment. These issues can be identified during the testing and verification process for the sample handling, manipulation and preparation equipment and informed decisions can be made then. Given that the most intense period of work will be during the Preliminary Examination period and for a time afterwards with many samples likely being prepared this is the period where the cross-contamination risk is perhaps greatest.

Some sample preparation may involve the samples being altered in some way, for example to produce a polished block the sample needs to be embedded in epoxy resin. It will be necessary therefore, to keep 'dirty' preparative techniques separate from cleaner areas of working. Once samples are prepared for study it can be the case that these samples will be reused many times.

The method of sample storage will be highly dependent on the type of samples returned from space. At a minimum, the sample storage environment should be controlled both in terms of environment (cleanliness, temperature, humidity, atmosphere, etc.) and access. In terms of environment there are different levels of control possible – the sample microenvironment, such as the environment within a single sample storage vessel (e.g. a tube, box, etc.) and the wider environment e.g. glove-box, cabinet in addition to room-level control. For such important

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specimens as those from sample return missions, it will likely be necessary to have such multi-level approach to storage, especially for particularly sensitive samples such as those that are frozen or which contain volatile components. Access to the samples needs to be controlled and monitored, both from a security point of view to prevent possible malicious activity but also as a way to ensure environmental stability. Sample storage environments should be monitored regularly using an electronic system with built-in alarms should any issues be detected. Samples that are returned from scientific study but which can be reused must be kept separate from those which are considered pristine.

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V. Robots versus humans

This section of the deliverable aims at discussing the use of robots versus humans, the advantages and disadvantages of robots compared to humans and possible improvement of the functional requirements of the ESCF.

Information has been obtained from meetings and discussions over the last months with a number of robotics experts, high-containment experts, curators of collections, and a variety of colleagues from different fields and expertise. In addition, information extracted from the WP3 Workshop, from previous EURO-CARES deliverables and publications as well as from unpublished reports, were used.

Although the list is not exhaustive, we should thank for the interesting discussions and information that they shared with us the following people (in alphabetical order): Judith Allton (NASA Johnson Space Center, Houston, USA), James "Sandy" Ellis (Merrick Canada ULC, Kanata, Ontario, Canada), Michael Hofbaur (Joanneum Research-Institute for Robotics and Mechatronics, Klagenfurt, Austria), Andreas Kurth (Robert Koch Institut (BSL-4), Berlin, Germany), Paul Langevin (Merrick Canada ULC, Kanata, Ontario, Canada), Cecilia Laschi (The BioRobotics Institute, Pontedera (Pisa), Italy), Peter Mani (tecrisk GmbH, Bern, Switzerland), Narendrakrishnan Neythalath (Joanneum Research-Institute for Robotics and Mechatronics, Klagenfurt, Austria), John Vrublevskis (Thales Alenia Space UK Ltd, Bristol, UK), and Ryan Zeigler (NASA Johnson Space Center, Houston, USA).

After the identification of all the main tasks that could be undertaken using robots and robotics systems in the facility, a questionnaire was prepared to structure discussions with the selected experts. The results of this survey is summarized here. It should be noted that efforts were mainly focused on specific tasks rather than on specific activities but it should be recognised that robots and automation in general can also be used for data collection such as recording sample movements inside the facility, data processing and many other applications that are not discussed in this report.

This study was conducted to allow the evaluation of advantages and disadvantages of using robotic systems compared to humans, knowing that the main objective in using robots is to improve the functional requirements of the ESCF and remove human sources of environmental contamination. The different arguments for and against the use of robots (versus humans) are presented here, leaving the reader free to decide by him- or herself which place to give to robotics in the ESCF.

The discussion of whether to use robots versus humans and/or both for some tasks to be conducted within the facility is discussed in the framework of the WP3 because of the direct implications it would have on the design of the facility.

1. Main tasks or applications for which robotic systems were considered

- Opening of the sample container.
- Extraction of the sample(s) from the container.
- (Micro-)manipulation of the sample(s).
- Transfer of the sample in the scientific instrument (for preliminary examination and characterization).
- Transfer of the samples to the storage room.

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Some of these tasks are highly dependent on a number of factors and parameters that were unknown or not defined at the time this report was compiled. In some cases, the applicability of the use of a robot can be greatly affected by these unknown factors and parameters. For example, the types of instruments to be used in the facility will affect the applicability of the use of a robot to transfer a sample in a yet undefined scientific instrument. This is even more critical for the (micro-)manipulation of the samples, without previous knowledge of their size, or how diverse and non-homogeneous they will be, their properties, etc.

Other tasks that would be conducted within the ESCF, such as the waste management process, were not considered in this survey.

2. Different types of robots and their suitability

Different types of robots can be defined:

- fully autonomous robot (i.e. capable of self-adaptation to the situation).
- autonomous robot programmed for a specific task (object and path planned in advance or at the time of carrying out the operation, based on the specificity of the task to be conducted).
- teleoperated robot (i.e. controlled by a human operator).

Table 3 summarizes how suitable or not suitable the different types of robots are in function of the different tasks to be conducted.

Type of robot Task	Fully autonomous robot	Autonomous robot programmed for a specific task	Teleoperated robot
Opening of the sample container	2	2-1	0 (or [1] in case it does not go as planned)
Extraction of the sample(s) from the container	0	0	2
(Micro-)manipulation of the sample(s)	0	0	1-2
Transfer of the sample in the scientific instrument	0-2 ([0] in case the sample is not fixed on/in a specific holder)	0-2 ([0] in case the sample is not fixed on/in a specific holder)	1-2
Transfer of the samples to the storage room	2 (automated storage and retrieval system)	1 (if automated storage and retrieval system is not used)	0

Table 3. Suitability of the different types of robots to operate different types of tasks (based on our questionnaire and the variable answers we received). "0" in case the robot is "not suitable", "1" in case it is "quite suitable" (or suitable under specific conditions), and "2" in case it is "suitable". Important to be noted is that in some cases, specific conditions can drastically change the suitability of one type of robot; in such a case some text is added.

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From the results of our survey, it appears that for robots the most difficult task to be conducted is the (micro-)manipulation of samples due to the number of uncertainties on the nature, size (range and homogeneity), properties, etc. of the samples to be manipulated.

The transfer of the sample in the scientific instrument for preliminary examination and characterization (if the samples are fixed/mounted on a specific holder) was felt to be a task that would be suitable for robots as would be the opening of the sample container and the transfer of the samples to the storage room. In the case of the transfer of the samples to the storage room, an automated storage and retrieval system (ASRS) was thought to be the best solution. ASRS are commonly used in many industrial sectors such as pharmaceuticals, warehousing and libraries, etc. (Figure 3). The advantages in using such a system are accuracy (tracks permanently the position of the samples and records all the movements), security (as humans do not have to access the storage area), possibility of working in extreme environments (such as at cold temperatures), and efficient use of storage space, etc.

Figure 3. Example of an automated storage and retrieval system (Source: Wikimedia Commons).

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3. Challenges and solutions

Robots and robotic systems would need to operate in a clean and high containment (forward and backward contamination) environment without shedding of particles from motors or joints. This is somewhat challenging, even if some solutions exist already. There are some robots working in cleanrooms, i.e. robots that were developed specifically to meet the requirements of clean environments (typically used by semiconductor companies; see e.g. Mathia 2010), such as:

https://www.robots.com/applications/cleanroom

http://www.staubli.com/en/robotics/6-axis-scara-industrial-robot/specialized-robot/cleanroom-semiconductor-robot/

http://www.kuka-robotics.com/taiwan/en/products/industrial_robots/special/clean_room_robots/

In most of these cases, appropriate coatings have been developed to be able to operate robots in clean environments. These coating materials are able to contain any leakage and can be sterilized (on this respect, coating materials of robots used for medical applications should be reviewed). However, these coating materials were developed to limit particulate contamination, but not molecular contamination. Certain coatings and lubricants may off-gas and produce molecular contamination. Cleaning can also be challenging (see e.g. Saito et al. 2017) and more work is necessary in that respect. To summarize, some solutions to adapt robots to the work environment inside the ESCF already exist but still an assessment on whether they are inorganically and organically clean enough need to be conducted. Cleaning protocols would also need to be defined and assessed.

In recent years, alternative (lighter) materials have been developed for the construction of robots (i.e. robots are generally constructed with aluminium, steel or titanium) including different types of composite materials and plastics. As the joints and motors are the main sources of contamination (i.e. friction creates particle contamination), one solution is to keep them partially outside of the working environment. The use of non-conventional robots may also be a solution, such as "soft robots" (i.e. robots characterized by non-conventional structures, constructed with soft and deformable materials like silicone, rubber, plastic, etc.). In general, soft robots are continuous deformable structures that do not have joints and have no motors on board. Such robots have a number of advantages over traditional rigid robots. Their deformable structures allow them to adapt to the environment and to some uncertainties; this could allow for example grasping and manipulation of samples with unknown/undefined properties (such as size or even consistency). However, they may be less suitable for high precision tasks. This field being relatively new (Shen 2016), most soft robots are right now only at the prototype stage but future developments should be highly considered for a usage in the ESCF. A good example of a recent and successful development is Stiff-Flop (STIFFness controllable Flexible and Learn-able Manipulator for surgical OPerations; a number of references are available here: http://www.stiff-flop.eu/index.php/en/). Reader can find more information on soft robots here: http://softrobotics.org/.

Apart from the contamination risk from moving parts and lubricants, one of the issues is the gripper itself. Developments are needed to obtain suitable grippers, in term of efficiency and of non-contamination (knowing that this task is complicated by the to some extent unknown nature

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of the samples and their properties). Currently, a number of different physical effects are used to guarantee a stable grasping between a gripper and the object to be grasped (in our case a sample or a fraction of a sample). They are four general categories of robot grippers (see e.g. Monkman et al. 2007), namely:

- Impactive (such as jaws or claws; physical grasping of the sample [not very suitable in the case of small samples])
- Ingressive (such as needles or pins; physically penetrates into the sample [not suitable in our case])
- Astrictive (suction forces are applied to the sample surface; whether by vacuum, magneto- or electroadhesion)
- Contigutive (requires direct contact for adhesion to take place; use of a glue, surface tension or freezing)

The purpose of this report is not to review all different types of robot grippers, their advantages and disadvantages, but this is something that would need to be done to be able to move forward if the task of (micro-)manipulation is to be robotized.

4. Cost of robots

Alongside with pros and cons and suitability of robots, costs need to be discussed, and would be a factor in their selection for the ESCF. Since the project is still at a theoretical stage, a cost estimate cannot be presented without the full requirements. However, knowing that there is no existing "off the shelf" robotic system that could be used in the ESCF, we can state that robots would have to be designed specifically for the ESCF, which means that development and design costs would have to be considered. The starting point could be an existing robotic system with an end effector (such as a manipulation tool or gripper). A thorough testing as well as cleanliness and biocontainment certifications should be then performed on-site. Service and decommissioning should be added to the costs. For the types of robots that would be used in the ESCF, costs would likely be 1/3 for "system costs" and 2/3 for "engineering costs". The use of specific materials would likely increase the total cost of production by a factor of 5 or even 10. In addition, the software part, allowing to use and control the robot, should not be neglected as this part is more prone to reliability issues than the hardware.

Two to three years seem realistic from the time the requirements are defined until the robot operates in the ESCF. However, this timing mainly depends on the requirements.

5. Current usage of robotics in curation facilities and BSL-4

Robotics in curation facilities

Currently robotics are not used at the NASA Johnson Space Centre curation facility in Houston (USA). The exception is a recent semi-automated micro-manipulator which is used for picking small cosmic dust grains (it is currently not done in a cabinet environment but rather on a flow bench). However, in the case of Mars 2020, the plan is to drill rock samples to, among other reasons, make them conform to shape and size to be handled robotically. This being said, past experiences in the use of robotics is limited to very few experiments such as the use of a robotic manipulator (i.e. a small robotic arm) in an advanced curation glove box (Bell et al. 2013).

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At the Planetary Material Sample Curation Facility (PMSCF) of the Japan Aerospace Exploration Agency (JAXA) in Sagamihara (Japan), micromanipulators are used for handling very small particles. They consist of a specially designed electrostatically controlled micromanipulation system which is operating in a ultra-pure nitrogen environment (Yada et al. 2014). [Theoretical information on electrostatic particle manipulation could be found in Saito et al. (2007).] They also constructed an electrostatically controlled micromanipulation system composed of commercial based instruments which could be used both in a clean booth of an electron microscope room and also in a glove box filled with nitrogen.

In addition to these limited use of robotics in existing curation facilities, a few studies on handling and (remote) (micro-)manipulation systems for extraterrestrial samples have been completed or are currently in progress, such as for example Stewart (2010), Nelson and Mani (2011), and Vrublevskis et al. (2016). However, on the basis of these studies, no concrete system was yet produced and tested. Some of the used requirements do not make any sense, such as for example in Nelson and Mani (2011): "[...] *a special handling system is required that will allow these tasks to be performed under Mars ambient conditions* [...]". A number of studies on facilities to receive, contain and curate extra-terrestrial samples while guaranteeing terrestrial planetary protection, called for the use of robotics. An example is in Beaty et al. (2009) where robots are either used for the entire spectrum of tasks to be conducted within the SRF, such as for the preparation, analyse, transfer of the samples, etc. or for only a subset of these tasks. In all these cases the use of robotics has direct implications on the concept of the facility.

Robotics in BSL-4

The use of robotic systems to handle pathogenic agents has been proposed as a way to increase the safety of BSL-4 facilities by reducing potential operator exposure. Robotic systems are widely used in microbiology laboratories (for diagnostic procedures) and in biotechnology/pharmaceuticals (for high throughput screening of antimicrobial compounds). However, due to high capital costs and economics they are only used when the sample throughput is very high. Because of the low incidence of highly pathogenic agents within humans and the additional capital burden of containment measures, and knowing that the conducted work is rather individual, robotic systems have until now not been used to any extent at high containment. Nevertheless, robotic systems have been considered for use in BSL-4 facilities as it would allow to separate any operator from the process. In that respect, a system is being developed in a european laboratory in which antiviral compounds screening is carried out using a robotic process line within a metal isolator. This device is being evaluated at BSL-2 but has been designed to operate at BSL-4 if required. For use in high containment systems any robotic system will have to withstand liquid and gaseous disinfection to prevent cross contamination of samples and allow servicing and maintenance.

(Micro-)robotic actors

No review of the current main (micro-)robotic actors (i.e. commercial players, research groups and institutes) is presented in this deliverable knowing that an extensive review on this topic was already presented in the report by Nelson and Mani (2011). However, to be added to this review would be the Institute for Robotics and Mechatronics (JOANNEUM RESEARCH Forschungsgesellschaft mbH; www.joanneum.at/en/robotics), located in Klagenfurt (Austria), which was recently created and is already a main actor in the field.

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To be noted is that this field is evolving so fast that new players and start-ups constantly appear on the market and, thus, any compiled list would soon be out of date.

Conclusions

In a number of previous reports about sample return curation facilities, robots have been recommended to replace humans for a number of tasks. However, it is not always clear what are exactly the advantages and disadvantages of the robots compared to the humans. Even if some of the advantages are obvious, a number of possible disadvantages are generally simply ignored or not properly considered. The interaction between humans and robots is also generally not discussed.

If we look at existing receiving and curation facilities, the use of robots is either extremely limited and in most cases not existent. The same is true for existing BSL-4 high containment facilities. However, in the few last decades, with the advancement in technology, the use of robots in industry has literally exploded mainly due to productivity and economic reasons and to the fact that some operations to be conducted need capacities that humans are not able to reach (i.e. in a number of cases robots outperform human performances). In our case, productivity and also economic reasons are definitely not the main decisive factors.

It is obvious that different types of robotic approaches can be used, all of them having some advantages and disadvantages depending on which perspective we look at them. In some cases robots are highly recommended, in other cases human is highly recommended, but in general it seems more appropriate to not consider both of them as separate actors as it is too often done, but the best option is likely to use a combination of both of them.

From our survey it seems evident that the main critical task for which novel solutions are needed is in the sample handling and micro-manipulation.

In general, a weak point for the use of robots is their limited flexibility knowing that flexibility was one important keyword that was defined in the deliverable D3.2 for the ESCF.

In conclusion, robots have a wide range of advantages and disadvantages, depending of the tasks to be conducted and the associated requirements and, it is certain that their utilization will increase with time and with the new technological advances to come.

Some of the challenges in the use of robots are the same as for humans, in particular in ensuring a clean environment.

Even if robots provide a number of advantages compare to human operators, in the case of sample return missions, being able to deal with the unexpected is one of the requirements ("we really don't know what we don't know"), and in such a case nothing is better than human. In other words, robotics definitely may have a place but not to replace humans. Accordingly, the concept of the facility should consider both, humans and robots, and integrate a close interaction between them in the facility.

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Final note for further robotic developments

In most (if not in all) cases, the object, in our case the sample, is "fixed" (i.e. not moving) and the robotic arm or manipulator is moving. Solutions taking into account that the sample can be moved should be considered as this would have some advantages (i.e. it would be too long to discuss the advantages here but this note is to suggest that it should definitely not be excluded or simply ignored).

Keywords

- In favour of the use of robots: reproducibility, highly suitable for dangerous tasks (i.e. safety reasons), precision and ability (i.e. do not tremble or shake as human hands), large range of operating conditions/extreme environments of usage, automatic tracking (less error prone and time saving), potentially less particle shedding.
- Against the use of robots: (very) task specific (not able to "handle the unexpected"; i.e. not able to accommodate as humans), limited (to no) flexibility, cost, lack of common sense/lack of human intelligence and judgment.

Interestingly, one of the motivations for the use of robots, i.e. to avoid contamination (forward and backward contamination), depending of the types of contaminants, play for but also against the use of robots.

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VI. Cleanliness and containment

1. Biological sterilization (restricted samples only)

Samples

There will be a requirement to sterilise any restricted return samples before they can go out of the ESCF for external studies, either before it has been proven to be free of lifeforms or if lifeforms have been identified. This is a prerequisite of the PP guidelines (COSPAR, 2002). Returned samples will be primarily composed of cored rock samples and regolith. If the sample presents a high porosity, which is the case for regolith, organisms might use pores and fissures as a microenvironment. This may only be determined through microscopic analysis of the rock, meaning it will need to be considered as contaminated internally until proved otherwise. Sterilisation of these rock and regolith samples will only be achieved by using an energy-based technology that can penetrate to their interior, such as heat or radiation. Validation will need to be undertaken to ensure that the appropriate parameters are met for effective sterilisation without altering the physical or chemical properties of the sample material and influencing future testing. More details on these methods are included in the D2.3 "Sterilisation and Cleaning" of the EURO-CARES project.

Waste

Solid waste decontamination

Autoclaves

Autoclaves have historically been used to provide an effective method of sterilisation of laboratory waste (Block, 2001). The most effective way of sterilising waste before it can leave a high containment laboratory is through autoclaving. Within a BSL-4 laboratory the autoclave is required to be double-ended, with interlocking doors. The external doors should only be able to be opened once a cycle has been completed to all of the parameter set points. This stops unsterilised material from being released from the laboratory.

The European standard 12347 describes the minimum operating parameters that must be exceeded for a correct autoclave cycle. There are a number of different autoclave cycles that can be used and these will reflect the waste that is being processed, i.e. high liquid volumes, or highly absorbent loads. Validation of the cycle can be completed using either chemical, physical or biological methods, or a combination of more than one. Spores of the bacteria *Geobacillus stearothermophilus*, are recommended as the biological indicator organism, as these are resistant to moist heat. Chemical indicators that change colour after exposure to the required conditions can be used, or using thermocouple recorders to establish the same conditions have been achieved are available to be used.

If the autoclave cycle has failed then the waste inside can be returned to the laboratory and the autoclave repaired. Autoclaving provides a well-established and easily validated methodology of sterilising waste generated in the laboratory, using monitored physical parameters.

Incinerators

Incinerators use combustion at high temperatures to reduce the waste within it to non-combustible ash. Incineration is used as the final stage of the solid waste disposal process, where waste is

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incinerated after autoclaving. As such there are no incinerators housed directly within BSL-4 containment facilities. The EU Directive 2000/54 stipulates that incineration must be used in animal BSL-4 facilities for the disposal of animal carcasses, although other technologies are being investigated as alternatives, such as alkaline hydrolysis. The use of incinerators is often highly regulated under national regulation.

The most standardised design of incinerator is the dual chamber incinerator. In this design the waste is fed into the bottom chamber which is operated at a temperature ranging from 870-980˚C, the oxygen content within this chamber is also regulated, allowing the control of the oxidation of the waste and fixing of the carbon. Waste gasses from this chamber are allowed to move to the second chamber, which is above the first one, where extra air is introduced to burn the waste gasses from the first chamber. The temperature in the second chamber is higher than in the first at >1093˚C (Block, 2001).

Generally, incineration after a validated autoclave cycle is unnecessary as there is little benefit and the incineration process is costly and environmentally unfriendly.

Liquid waste

The majority of the liquid waste produced within a BSL-4 facility is from either positive pressure suit decontamination showers or from personal showering. Small volume processes completed in the BSL-4 facility will also be collected in the effluent system. The effluent system must have two HEPA filters in series if it uses atmospheric ventilation to stop any contamination within the gases from being released in the environment (Chosewood and Wilson 2009).

The effluent treatment system must be completely sealed to prevent any leakage of effluent. Special measures need to be put in place for high containment facilities handling large animals, such as being constructed to be gas-tight at a pressure of 1KPa (Barbeito et al. 1995). Large animals cannot be contained within primary containment such as cabinets, so the room is treated as the primary containment. The animals produce a large quantity of potentially contaminated waste that cannot be completely collected and autoclaved such as within a non-animal high containment facility and therefore the effluent treatment system must be designed to a higher standard. The material the effluent treatment equipment is constructed of must be able to withstand any chemicals that are used in the treatment process and also used in the laboratory. Currently the preferred method of treatment for the effluent is heat, produced by steam, this is because it is easier to validate, control and therefore reproduce (WHO 2004). Other methods can be used such as chemicals or heat and chemicals in combination. After treatment the effluent can be cooled and discharged to the main sewer (HSE 2009), or if the treatment is chemical then the effluent must be neutralised and/or returned to a neutral pH prior to discharge. The construction of the effluent system should allow for regular inspection of the pipework, with no pipework being hidden from view e.g. by enclosures. Any drains and U-bends incorporated into the system must be able to be sealed or engineered to prevent drying out to stop any air from the effluent treatment plant from returning to the laboratory. The pipework should be able to be sterilised *in situ* to reduce the need for human intervention during operation.

The effluent system should work on a gravity feed because this removes the need for back up devices in the event of a pump or power source failure. The treatment vessels must be situated in a plant room that is bunded, to contain any leaks or spillages from the treatment vessels. This bunding must be able to hold the capacity of the treatment vessels plus an additional 10% (HSE

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2009). This needs to be demonstrated by filling the bunding with water to this level and then being held over a period of time with no loss. In larger facilities that are envisaged to be in continual use then it may be appropriate to use two processing tanks as once one reaches the fill level and the treatment is undertaken the other tank allows the laboratory to continue to operate. The processing tanks should be able to continually stir their contents to maintain homogeneity of the effluent during treatment.

Validation is completed using biological indicators, such as spores of *Geobacillus stearothermophilus*. After validation, monitoring of the physical parameters can be completed to determine if the process if effective, prior to release of the treated effluent to drain.

Tools

Decontamination of tools if they are thermostable will be completed either by sealing within an autoclave pouch and then autoclaving or, if thermolabile, treated chemically, either gaseous or liquid. If the tool cannot be decontaminated then single use tools can be procured for the facility. It will be important to identify and validate the selected technique in appropriate conditions.

Rooms

The surfaces of the laboratory and rooms will initially be treated using a liquid disinfectant to remove the gross contamination from them, often after this as the final step in the decontamination process a gaseous application of a decontamination chemical will be used. For this process there is a range of techniques and technologies that are available for use, e.g. formaldehyde, chlorine dioxide and hydrogen peroxide (Beswick et al. 2011). More details on these processes are found in the D2.3 "Sterilization and Cleaning" of the EURO-CARES project.

Showers for suited workers

On exit from a suited BSL-4 facility the exterior surfaces of the suit must be decontaminated before it can be removed. As all the sample handling procedures will have been undertaken within primary containment then any contamination should be at a low level on the suit. The showers should be linked directly to the laboratory exit via airtight doors. The shower will be at a positive pressure to the laboratory but at a lower pressure to the changing area to create a flow of air into the laboratory away from the areas where personal protective equipment (PPE) is not necessary. There are a number of different choices for the shower design with arrangement and type of shower nozzles that can be used. Showerheads that become clogged easily should be avoided, the National Institutes of Health (NIH) facility in the USA use shower heads that can be detached and cleaned easily to stop them becoming clogged (Crane et al. 1999). The effluent from the showering process is collected in the effluent treatment facility.

At present there is no defined guidance for the type of chemical that should be used in the decontamination shower or the cycle parameters (Klaponski 2011). Whilst the facilities can choose their own respective chemical, the introduction of the EU Biocide Directive will limit those that can be used in the future. For example, Microchem which is used in some USA and Canadian facilities cannot be used in Europe. During 2010, PHE received information from a range of BSL-4 facilities around the world on their shower cycles, this is shown in the Table 4 below.

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Facility	Chemical cycle (L)	Rinse cycle (L)	Total volume (L)	Type of detergent/disinfectant
A	68	160	228	Microchem
B	10	35	45	Microchem
C	60	30	90	Desintex
D	10	35	45	Microchem
E	10	35	45	Microchem
F	10	35	45	Microchem
G	33	60	93	Microchem
H	24	115	139	Microchem

Table 4. Shower cycles used in BSL-4 facilities.

The shower cycle decided for a new facility will need to be validated to determine its efficiency. A study completed by PHE investigated a shower cycle using Desintex against two different positive pressure suits (Chemturion, ILC Dover and BSL-4 suit, Honeywell) and the contamination positioning on the suit. The study found that increased removal from the suit was facilitated by using a brush, the removal from the different suits varied, and that the positioning of the contamination on the suit caused variances in the removal levels. This indicates that there is no set showering regime for use in the facility and different cycles will be developed on the choice of suit, potential level of suit contamination, work being undertaken and shower/nozzle delivery system used.

Redundancies are built into the shower set-up where a gravity fed tank of chemical disinfectant is always available so the suits can be decontaminated in the event of a power loss. This emergency tank needs to be large enough to wash and decontaminate the maximum number of suits that will be in the laboratory at any one time.

In both types of BSL-4 facility (cabinet line and suited) the operators will be required to also pass through a personal shower before exiting the laboratory. This will follow the removal of the positive pressure suit or in a cabinet line laboratory after removal of the individual's scrubs. Effluent from the shower after cabinet line, isolator or animal facility is treated in the effluent treatment system before discharge.

2. Protection of workers and samples (restricted samples only)

At the highest level of biological containment, BSL-4, there are two engineering approaches that are generally used for the safe handling of the high consequence pathogens. These are either:

- **Cabinet Line Laboratory**. Work is carried out within a series of interconnected class 3 Microbiological Safety Cabinets (MSC) where the worker uses gauntlets on the side of the cabinets to manipulate the infectious material,
- **Suited Laboratory.** Workers wear a positive pressure suit. Within a suited laboratory class 2 MSC are used to confer extra protection to the worker and the samples from contamination.

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This section will detail the two different laboratory types and how they could be applicable to a sample return facility.

Microbiological safety cabinets and cabinet line laboratories

There are three different types of MSC used in microbiology laboratories, classes 1, 2 and 3. Each of these classes use a combination of directional airflow and high air change rates to prevent exposure of workers to any microbial aerosol within the working area of the cabinet. Both class 1 and 2 cabinets are open fronted and require the worker to directly insert their arms into the cabinet to handle the samples, whereas the class 3 cabinet forms a physical barrier between the worker and the samples. Within Europe, cabinet performance is specified in the European standard EN 12469:2000 for biotechnology performance criteria for microbiological safety cabinets (BSI 2000).

MSC3

The MSC3 is designed to offer the highest level of protection to the worker and the surrounding environment whilst also protecting the work from particulate and biological contamination (Chosewood and Wilson 2009). The cabinets are generally constructed with a front window made of clear Perspex or safety glass and an airtight seal, which is held in place using locking nuts to the carcass of the metal cabinet. The glove ports, through which the sample manipulation occurs, are either through the window or below the window through the cabinet's body. Whole arm length gauntlets are secured to the gloves ports. The gauntlets are usually constructed of rubber but can be made of other material as long as it confers the level of protection and dexterity required by the users. Figure 4 shows a MSC3 cabinet in operation at PHE (Porton, UK).

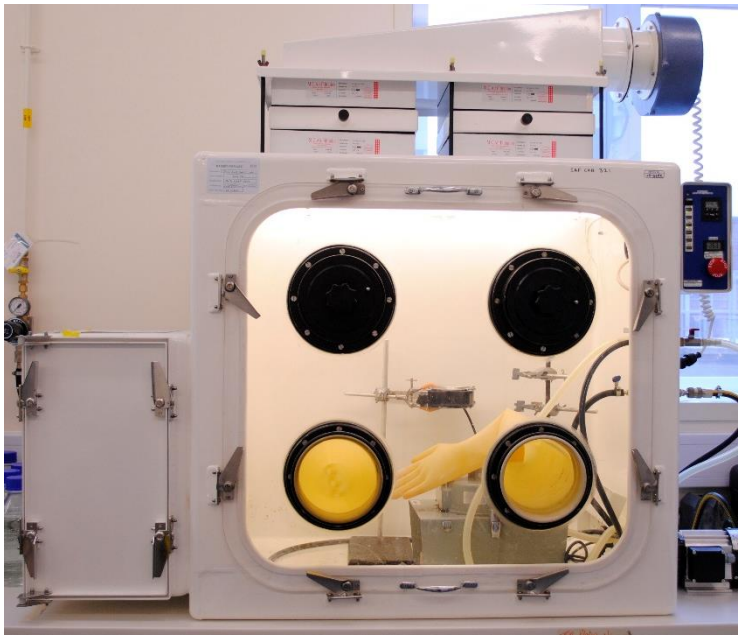

Figure 4. A MSC3 in operation at PHE Porton (UK).

An inflow of air is drawn through a single HEPA filter, passing over a baffle plate ensuring the air is mixed thoroughly within the cabinet. Air is then drawn out of the cabinet through double HEPA filters by a fan unit positioned after them. The fan unit operates at a higher flow rate than can be drawn through the inlet HEPA filter to ensure negative pressure is maintained within the cabinet. A number of cabinets can be linked together to form a cabinet line (discussed in more detail below),

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this provides a greater flexibility of sample processing tasks than could not be performed in a single MSC3. But for smaller operations, a single MSC3 can be used. Some of the MSC3 will have a pass box or other transfer systems to allow samples or equipment to be removed or to enter the cabinet during operation. Barbeito and Taylor's (1968) experiments during the 1960's on the protection afforded by an MSC3 in operation and showed that the cabinet was effective at containing an aerosolised bacterial challenge. Further experiments also proved that when the gloves were removed from a cabinet in operation, it could still provide a high degree of protection (Barbeito and Taylor 1968). The MSC3 should be operated at less than a negative pressure of 250 Pa with a minimum volumetric inflow of air of 0.05 m^3/s for each cubic metre of cabinet volume, as specified by the standard BS EN 12469.

The MSC3 exhibits a very high protection factor when operating correctly and allows the users to work without the necessity for respiratory protection or constraining suits. But the cabinets can be difficult to work with and restrictive due to the positioning of the glove ports. The high airflow and turbulent environment is unsuitable for handling some materials such as powders.

Cabinet line laboratories

Cabinet lines are made up of a number of MSC3 that are connected together to form a spine. From this spine, further MSC3's are connected and will be used for manipulation of the samples and to house the specialist equipment that is required for processing, e.g. microscope, analysers, etc. The cabinet line spine is used to move the samples along to the necessary cabinet branch where the manipulation/analysis can be performed.

Material entering a cabinet line in a BSL-4 facility will be passed through a dunk tank filled with a validated liquid disinfectant. The sample container must therefore be waterproof to avoid ingress of the disinfectant. The container will be immersed for a defined period of time before it is then transferred to the cabinet line and opened. At the terminal end the cabinet line will be connected to a double sided autoclave, which is used to sterilise any material that is removed from the cabinet line. Live samples, or other material that cannot be autoclaved can be packaged and either passed through the dunk tank or fumigated before removal from the cabinet line, rather than being autoclaved.

Testing of cabinet lines are not included within the European standard EN 12469, as this only details the tests required for single MSC3 cabinets, but the general principles can be used for cabinet lines. This will need to be discussed and agreed with the host country's regulatory agency.

The use of a cabinet line can be restrictive for the workers, but training can be completed more quickly than for a suited laboratory (Hilliard et al. 2007). This is because workers progressing from working in lower containment levels to BSL-4 will already have experience working with cabinets, but the majority of workers will not have previously worked with the positive pressure suits used. Operators working within the cabinet line are required to wear specific laboratory clothing that consists of disposable underwear and operating theatre scrubs with a lab gown. These will be removed on exit prior to a personal shower and then autoclaved before washing outside of the laboratory.

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Open fronted cabinets

MSC2

Class 2 MSC (MSC2) are designed as open fronted cabinets that gives protection to both the worker and the sample from aerosols. The cabinet operates by drawing air in through the open front, this then drawn downwards through a grill and passed through an internal HEPA filter, by a fan unit, before being recirculated to flow down over the work surface and form a barrier curtain of air over the cabinet opening. This protects the worker from any aerosol within the cabinet working area and protects the samples from contaminated laboratory air by passing clean air over them. Manipulations of samples are performed by the workers by placing their arms through the cabinet opening. These cabinets are often used when sample protection is required but the containment level of the material is low and a greater degree of freedom is required compared to a MSC3, e.g. tasks using cell culture. They are also used within suited BSL-4 laboratories for manipulations that are likely to generate aerosols, this limits any potential contamination to the cabinet and to the wider laboratory. They would potentially be used for BAP work in suited laboratories.

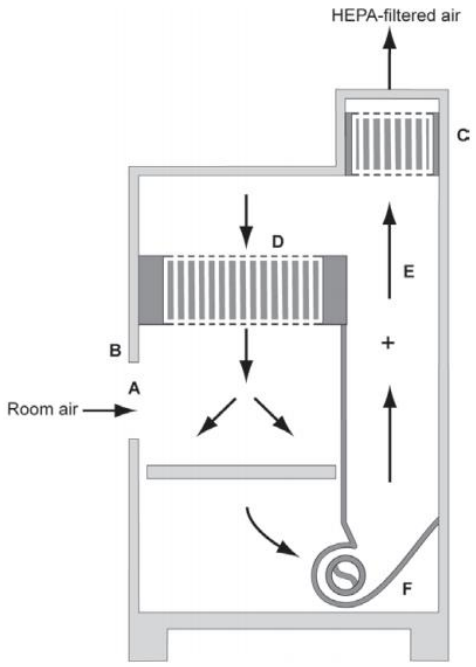

Figure 5. A Class II, Type A1 MSC. (A) Front opening, (B) Sash, (C) Exhaust HEPA filter, (D) Supply HEPA filter, (E) Control plenum and (F) Blower unit. The arrows indicate the direction of the air movement. Reproduced from Chosewood and Wilson (2009).

MSC2 cabinets should be tested against the European standard 12469. Using the methods defined within the standard, the MSC2 cabinet should achieve a protection factor of greater than 10^5. The operator protection factor is the ratio of particles that the worker is exposed to from a procedure within primary containment, to the level of particles that is sampled outside of the containment. This allows testing of the containment equipment to determine if it meets the required standard (Steward and Lever 2012). It has been demonstrated that protection factors of up to 10^7 are

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achievable (Stuart 1999). MSC2 can be sealed for fumigation by either closing the sash window or by attaching the night door.

MSC2 have been safely used at BSL-4 for a number of years but there is a lack of published data on the impact of workers undertaking manipulations in suits within MSC2. There is a potential for the contamination of the worker's gloves and arms, and without effective decontamination, contamination could be transferred from the cabinet to elsewhere in the laboratory.

Whilst effective when operating correctly, there are a number of different factors that can cause a reduction in the operator protection factor of an MSC2 and therefore the potential release of aerosols from it to the laboratory.

Airflow directed over the face of the cabinet

A major consideration in the set-up of a MSC2 is its positioning within the laboratory (Osborne and Durkin, 1991). External air movements over the open face of the cabinet can disrupt the air curtain used to both prevent aerosol release and provide protection to the sample (Osborne et al., 1999). Tests completed found that increasing the velocity of the air over the face of the cabinet from 100 feet per minute decreased the operator protection factor, logarithmically (Rake, 1978). Drafts such as this can be caused by heating, ventilation and air conditioning (HVAC) inlets and outlets, or the exhaust from positive pressure suits. To avoid this, the inlet and outlet HVAC vents can be modified to direct any airflow away from the cabinet face (Clark et al. 1990). A higher inflow of air into the cabinet also conferred an increase in operator protection factor (Macher and First 1984).

Incorrect operating practice

Workers must be trained to operate within a MSC2 without generating and then causing release of aerosols from the cabinet. The workers must have their arms within the cabinet to perform the laboratory procedures and with incorrect movements aerosols from within the cabinet can be drawn out on the removal of the worker's arms due to the turbulence caused by this motion (Macher and First 1984). It is important to perform work within the centre of the cabinet, working towards the rear of the cabinet can draw the worker closer to cabinet face and their body will disrupt the inwards airflow, causing air from within the cabinet to be released (Bennett et al. 2005). Therefore it is important to train the operator where to sit and how to work within the cabinet to avoid the release or ingress of aerosols whilst performing tasks. Training times will vary between worker and the duration can be judged by competency assessment.

Overall, when a MSC2 has been set-up and tested correctly then they will perform to the same operator protection factor as a MSC3 cabinet. There has of yet been no published studies investigating the aerosol release from a MSC2 when an operator is wearing a positive pressure suite. To maintain the cabinet's protection factor, it is important to train the workers in the appropriate techniques to avoid aerosol generation from the tasks and the subsequent release through poor working practices.

Flexible film isolators

Flexible film isolators (FFI) have been used in the UK for working with risk group 4 agents and infected small animals (van der Groen et al. 1980). FFI are thought of as non-standard MSC3, where a metal frame is constructed with a flexible canopy covering it. It increases the flexibility of

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the work that can be completed within the FFI compared to a MSC2 or 3 because the design can also include a number of half suits on the floor of the isolator allowing operators to be inside, increasing the usable surface area (Figure 6). There are glove ports around the exterior of the isolator allowing for the operation of larger pieces of technology. FFI usually operate at a significantly lower pressure differential and air change rate than a MSC3 cabinet, this can be changed depending on the use of the isolator. A fan unit, with battery back-up, is used to generate the pressure differential and air changes within the isolator. Bennett et al. (2005) have shown that the isolators can achieve a high degree of protection, similar to MSC2 and 3, even when gloves are removed or canopies ripped.

Figure 6. A solid frame isolator used in a BSL-4.

Modified FFI have been used for the transport and treatment of infected patients with high risk group agents, such as during the recent West African Ebola virus epidemic, where infected workers were transported by plane to specialist treatment facilities and then housed in large isolators during treatment.

Cabinets provide a high degree of operator and product protection. They can be adapted to the specific procedures that will be carried out within them using different arrangements, lower inflow of air, ultra HEPA, different gauntlets, but they do have limitations on the dexterity and size of equipment that can be placed within them. FFI have been developed to provide a solution to these problems by the use of half suits, the workers can operate from within the isolator in conjunction with those using gauntlets on the exterior surfaces. Flexibility of use can be incorporated into a cabinet line by future-proofing it with the design of sections where further cabinets can be attached containing additional equipment. This allows the modification of operations performed within it as newer equipment becomes available.

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Suits

Positive pressure suits are used within all BSL-4 laboratories, except the UK where the regulating body required the construction of cabinet line high containment laboratories when the facilities were built, as primary containment for the worker as opposed to a MSC3 cabinet line or flexible film isolator. With the operator wearing the positive pressure suit specific procedures with the infectious agent will be undertaken in a MSC2 to reduce the possibility of release to the wider environment because suits will only help to protect the worker, not the laboratory environment. However, for non-normative process such as large animal experiments, suits can be used as the main containment system. Positive pressure suits have been used within the nuclear industry with a long history of safe use.

There are currently two main manufacturers of fixed gas line positive pressure suits for use in BSL-4 laboratories; ILC Dover (USA) and Honeywell (France) (Walker et al. 2011). Positive pressure suits have an airline fitted to the suit through which breathing air is supplied, either from bottles or air compressors. The air is generally supplied into the suit from a detachable air line through a valve incorporating a HEPA filter on the outside and through a noise reducing mechanism. The suits incorporate a number of one way exhaust valves which are situated beneath splash covers. The airflow into the suit is higher than that leaving it, so the suit becomes positively pressurised and inflates. The positive pressure of the suit to the laboratory environment is one of the mechanisms that confers resistance to the user, by stopping the ingress of aerosolised particles, the other is by the physical barrier given by the suit.

Although the operation of the two main suit types is similar, there are large differences between them. The Honeywell BSL-4 suit operates at a higher airflow rate, 470-950 L/min, than the ILC Dover Chemturion suit, 142-155 L/min, meaning that a more powerful compressor is required for operation, especially if there is more than one Honeywell suit being operated at the same time. During emergencies, if the compressor fails, then, often a compressed air bottle back-up system is employed, again meaning that more bottles will be required with the Honeywell suits.

The suits are manufactured using different materials which can be affected in different ways by the disinfectant chemicals (Kümin et al. 2011) and should be investigated prior to use in a facility. The Chemturion suit is made of durable chlorinated polyethylene (Chloropel™), which is a blue colour and more rigid that the Honeywell suit. The Chemturion has a large clear forward facing visor made of polyvinylchloride (PVC). The Honeywell BSL-4 suit is constructed of a polyester fabric coated with PVC, with the panels sealed using high frequency welding. This provides a lighter weight suit. Again the suit has a clear visor, but this is completely around the head of the wearer. The suits can be seen in Figure 7. Whilst the Chemturion suit is supplied in three sizes, small, medium and large, the Honeywell BSL-4 suit can be made to the wearer exact dimensions. This means that whilst a single Chemturion suit can be used for multiple workers the Honeywell BSL-4 suit will only be suitable to a specific individual (unless a number of people have the same measurements).

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Figure 7. A Honeywell BSL-4 positive pressure suit in a decontamination shower mock up.

The operator protection factor of positive pressure suits has been investigated by a number of different laboratories (PHE, UK and Spiez, Switzerland). The work completed showed that both suits conferred a high level of operator protection factor to the user (Kümin et al. 2011; Steward and Lever 2012). Testing of the suits showed that under extreme movements it was possible to briefly negatively pressurise the suits and force some air inwards to the suit through the one way valves but this was for a short duration and the ingress of aerosolised particles was very low. This ingress can be eliminated through correct training of the workers to use smooth steady movements.

Working within those suits can be difficult, with the extra weight of the suit resting on the shoulders of the worker. The temperature of the incoming air needs to be carefully regulated to avoid the overheating of the worker. The noise of the incoming air can also be an issue for long duration use of the suit and communication. The Chemturion suit has a higher noise level within the suit compared to the Honeywell BSL-4 suit, but both usually require the worker to wear hearing protection when used for extended periods of time (Steward and Lever 2012). The noise within the suits also makes communication difficult between workers in the suits and to outside of the laboratory. Push to talk radio systems and head units are used within the suits. This also allows workers external to the laboratory to contact those within it in an emergency case.

Regular inspections and tests are undertaken on the suits to ensure they operate effectively. Visual inspections involve monitoring of the welds and zips prior to use of the suit, which the suit is physically tested using a pressure hold test to ensure there is no microscopic damage to it, using the European standard EN 464:1994.

The layout of the laboratory needs to allow the manoeuver of the worker in an inflated suit without danger of knocking into any equipment or damaging the suit.

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Workers attire

Within both cabinet line and suited BSL-4 laboratory, safe operating procedures include that the workers must remove their own clothes prior to entry into the laboratory (Hilliard et al. 2007). Within the cabinet line laboratory, disposable underwear is worn under operating theatre style scrubs, which are then covered by a rear fastening gown (solid front). The clothing worn in a suited laboratory will depend on the operator's preference. Within both laboratory types, the only personal item that can be worn is glasses knowing that on exit they must be washed in the personal shower with the worker. On exit of the laboratory, the clothing will be removed and either disposed of via incineration or sterilised using an autoclave, if reusable.

Gloves

The main interface between the worker and the infectious material being handled in a BSL-4 facility is either gauntlets in a cabinet line laboratory or gloves on a positive pressure suit. Before entering the laboratory, the worker will always put on a set of personal gloves. The gloves attached to positive pressure suits will be made of neoprene or heavy duty household cleaning gloves, where the gauntlets on the cabinet line are generally made of rubber, although other materials with better disinfectant compatibility are now available. Therefore, in each laboratory there are two layers of protections between the potential contamination and the worker's hands.

Biological laboratory gloves are usually manufactured from either latex or nitrile and are useful for a secondary barrier if used correctly (Mansdorf 1987). Training must still be given to the worker in good laboratory practice as any contamination on the exterior of the gloves can still be transferred to other surfaces or the worker's face with poor practices similar to if no gloves were worn at all.

Gloves can be affected by the disinfectants used for decontaminating a laboratory, leading to permeation and penetration of the gloves by the infectious agent. A number of studies have been undertaken to identify the chemical agents that can permeate gloves, and European standards have been produced e.g. BS EN 374-2:2014 Protective gloves against dangerous chemicals and microorganisms, determination of resistance to penetration. Alcohols can penetrate a range of glove materials, one study showed that alcohol was detected within the gloves tested after 10 minutes exposure (latex, nitrile, and a synthetic polymer) (Baumann et al. 2000).

Perhaps one of the major criteria for selection of gloves is worker dexterity. Different materials can have an effect on the dexterity of the worker. In a comparison between latex and nitrile gloves there was a slight decrease in fine dexterity movements when workers used nitrile gloves, but during gross dexterity testing no difference was detected (Sawyer and Bennett 2006). The dexterity of the worker is further decreased when a secondary layer of glove is worn (increasingly so with the thickness of the second layer, i.e. rubber gauntlets).

These points show that there are a number of options for use for glove materials in the facility, for both primary and secondary barriers. Careful consideration needs to be given as to what processes will be undertaken and then the most appropriate gloves can be chosen for this process. For example, if a chemical cleaning is used, then, the gloves material will need to be tested against the chemical to determine if they are compatible. Whilst a material might provide excellent dexterity, if they are prone to breakage or become easily permeable, then an alternative option might need to be sorted that decreases dexterity but provides more protection.

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Containment Design for ECSF (restricted)

The overall design for the use of primary containment within the facility will firstly depend on the type of mission (restricted or unrestricted return) that will be handled in that area. Unrestricted missions can use similar designs and technologies that have been used for previous missions i.e. Hayabusa, Genesis, etc. These will just require the sample to be maintained in a sterile and inert atmosphere as possible to reduce the effects of Earth contamination on the samples to a minimum.

Restricted return missions will require different containment techniques through the facility. It will be a requirement to maintain the sterility of the samples whilst also ensuring there is no release of the sample to the laboratory or the environment. For the removal of the samples from the Earth return capsule (ERC), workers could wear positive pressure suits to protect themselves from any sample contamination if there had been a non-nominal landing and ERC containment failure. This could also be accomplished using a flexible film isolator or cabinet line with direct human manipulation, or using a double walled isolator and remote manipulation. The set up of the facility could be dependent on the ERC design and the size and complexity of the sample removal could lead to multiple designs.

As with the cabinet line, once the restricted return sample enters the facility it will not leave containment until it has been sterilised or proven not to contain lifeforms. Therefore, the easiest approach would be to keep the samples within primary containment within the rest of the facility, this will also allow for an environment to be kept round the sample that will avoid its contamination and fulfil the planetary protection policy of reducing the probability of any sample release.

Secondary lab containment

High containment laboratories are designed and constructed to decrease the risk of an infectious agent being released to the wider environment. The first method for this is the primary containment used; either positive pressure suits or MSC. The next level is the secondary containment of the laboratory, some aspects of which are the negative pressure, directional airflow, sealability and filtration of extract air.

Negative pressure

Each high containment laboratory has a set of design requirement and these will vary greatly between the laboratories. Negative pressure is stated as a requirement in a number of guidance documents that have been produced by regulators around the world, but often no specific recommendations are made and the set points vary greatly (Rogers et al. 2007; Ide 1979).

For example, in the UK, the Health and Safety Executive requires a BSL-4 facility to have a minimum pressure differential of at least -75 Pascals between the laboratory and the ambient environment when handling specified animal pathogens, where the Advisory Committee for Dangerous Pathogens state there should be a pressure cascade of -30 Pascals for each containment layer (HSE 2009). Other regulations around the world recommend different figures and this can be seen by the pressures used in those facilities:

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- The BSL-4 laboratories built and used by Public Health Canada use a series of four airlocks with a difference of 50 Pascals between each one (Crane et al. 1999).
- In the high containment facility in Geelong, Australia, increments of 100 Pascals are used between facility sections (Crane et al. 1999).
- The National Institute of Health, USA, employs a negative pressure of 50 Pascals in the facility shell, with a further reduction of 12 Pascals for the suit entry and laboratories (Crane et al. 1999).
- The P4 facility in Lyon, France, uses final pressures of -40 Pascals in the entry room, decreasing to -90 Pascals in the animal facility autoclave room (ABSA 2002).

The use of high pressures within a facility needs to be balanced with the operation and functionality of the facility. The facility will need to be built to withstand high pressure differentials and this can add cost to that facility in terms of building quality and in the energy consumption of the facility when running. Achieving the desired pressure differentials can be challenging and can be the cause of delays caused by lengthy commissioning periods.

However, there is little evidence for the use of complex pressure cascades or high pressure differentials increase aerosol containment. A study was completed, investigating different negative pressure cascades in a mock up BSL-3 laboratory. The laboratory protection factor (LPF) was measured to define what the ratio of contaminant from the laboratory to an uncontrolled corridor was outside. It was found that there was no correlation between the LPF and the magnitude of negative pressure when the lab door was opened. But there was a relationship between the inflow of air and the LPF, an increasing inflow rate of air conferred a higher LPF (Bennett et al. 2005).

Air change rates

Within the laboratory, air change rates are calculated to remove the laboratory heat load and to reduce the concentration of contaminants within the laboratory. It is often thought that a high air change rate in the containment laboratory is sign of good performance, but there is little evidence as to what level of air change rates should be achieved in a BSL-4 facility.

In a functioning laboratory, any aerosols produced will be contained within the cabinets where the work is completed. If there is a release outside of the cabinet then the worker will be exposed immediately and even with a high air change rate (20 air changes an hour) it will take 14 minutes to remove 99% of the aerosols from the laboratory. As mentioned previously, an increase in air change rates will cause more ingress of air to the laboratory and a decrease in the protection factor of open fronted cabinets. Therefore, careful consideration needs to be given as to the necessary air exchange in the SRF. But this might need to be high depending on the heat load of the equipment in the facility.

Pressure tightness of the laboratory

Pressure tightness of the laboratory is important for a number of reasons. Setting a leak rate from the laboratory can be used to define the build quality of the facility and to define a standard value that can be used for testing. The leak tightness of the laboratory can also help to prevent the uncontrolled release of gaseous decontaminants during fumigation.

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Pressure testing of the facility can be carried out by pressurising the required area to a set point and measuring the rate of decay over a defined period of time. This can be done at positive or negative pressure. But this testing method does not accurately reflect the natural leak rate of the laboratory as the area is pressurised to a much higher level than would be seen in normal operation.

Laboratory filtration

High Efficiency Particulate Absorption (HEPA) filters are used in different laboratory types to prevent the release of particulate matter from containment. HEPA filters were originally designed for use in the nuclear industry (Abraham et al. 1999). HEPA filters are produced from one continuous sheet of filter medium that is then folded, with the folds being separated to avoid them touching. This folded filter medium is then bonded into a filter housing which has a gas tight seal running around the outer edges (First 1998). Usually the HEPA filter is sealed in place using clamps and this requires compression of the seal to about 80% for a leak proof finish. HEPA filters capture airborne particles in one of three ways:

- Impaction (particles >1 μm), larger particles will impact onto the filter fibres as opposed to following the air currents around the fibres. The impaction factor will decrease with increasing airflow or greater distance between fibres.
- Interception (<1 μm), small particles are drawn along the air flow path and contact the outer surface of the fibres and captured.
- Diffusion (<0.1 μm), the smallest particles that are under the influence of Brownian motion will contact the fibre and adhere to it. The diffusion capture process increases with low flow rates through the filter.

As a general rule once a particle has contacted a fibre, it is attached via van der Waals forces and is not released (First 1998). The size of the particle most likely to penetrate through the filter is approximately 0.3 μm. But this will be dependent on the velocity of the air passing through the filter.

Filters are rated on their performance to stop particles of 0.3 μm passing through them. In Europe there are 7 classes of HEPA filter according to the European standard EN 1822. The qualifying standards for HEPA filter testing were developed when HEPA filters were first produced and modern production means that the majority of the filters exhibit greater performance, providing a buffer for the filter to the expected standard.

Newer designs of HEPA filters are being developed, using PTFE membranes, and this may be an advantage for the ESCF as it would reduce the possibility of fibre shedding into the working area.

The EU Directive 2000/54 requires the air supplying a high containment facility to pass through at least one HEPA filter, and two HEPA filters in series on the extract, but mounted separately. This allows the independent testing and replacement of each filter (HSE 2009). Once a filter is installed it still requires *in situ* testing to ensure it is operating correctly and identify if there have been any issues in the transport of installation of the filter. To make the testing and replacement of the extract HEPA filters easier they are usually located in the plant room outside of the laboratory. HEPA filters installed should be regularly tested to ensure they are continuing to operate correctly, presently in the UK the regulators require testing to be performed every 6 months (HSE 2009).

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The use of double HEPA filters is designed to provide protection if one of the filters ever failed. The guidance for this was written over 30 years ago when the filter production techniques were not as good as the current methods. A review of the reasons for HEPA filter replacement in the Australian Animal Health Laboratory, Geelong, in 1999, found that the reasons for replacement changed over the course of 13 years. From a mixture of defects in the filter medium, failures of the gaskets, and blockage of the filter material at the beginning of the study and in conjunction with the methodology for producing HEPA filters improving the major reason at the end of the study was due to blockages of the filters (Abraham et al. 1999).

The use of double HEPA filtration for the extract of BSL-4 laboratories can be seen as above what is necessary. All aerosol generating procedures are undertaken within safety cabinets that themselves are double HEPA filtered. Therefore it is difficult to envisage any procedure that, barring intentional aerosol generation, will produce enough particles to penetrate one, let alone two HEPA filters. Within a ESCF this would also be the case and an argument could be made to decide on the number of HEPA filters on a risk assessment basis for each of the laboratories, depending on the procedures that will be undertaken in them and the likely challenge to the filters.

Recirculation of air

Recirculation of air from cabinets is allowed within the EU Directive for BSL-4 facilities, but other guidance (WHO 2004) only allows recirculation in a suited laboratory after double HEPA filtration. No rationale is given why recirculation is not permitted in a cabinet line laboratory.

The recirculation of air from the cabinets within the facility would require careful consideration, justification to the regulating body and will be dependent on the area within the facility. For example in the curation facility where an inert gas will be used to maintain the environment within the cabinets then this cannot be recirculated into the room as staff members would not be able to breathe and therefore external ducting would be required.

Air showers

Air showers are airlocks separating rooms of different cleanliness levels, with an active removal of particles and air filtration. They have interlock systems, meaning that on the way into the cleaner area, once inside the air shower, the worker cannot exit before the scrubbing process (30s to 120s) is completed. On the way out, air showers are generally no used, and serve only as airlocks. Shape of an air shower can vary, from a small cubicle with two or three doors, to a tunnel for larger equipment to be brought into a cleanroom. They can be equipped with a static ionization system, which might prove very useful for laboratories handling small particles.

Depending on the facility, air showers were present (Hayabusa Curation Facility, JAXA; Stardust Curation Laboratory, NASA; etc.) or not (Genesis Curation Facility, NASA; Hayabusa Curation Facility, NASA; etc.). Based on the interviews we conducted, the usage or not of air showers is based more on customs and knowledge of the facility manager rather than on comprehensive studies of air shower efficiencies. However, the facilities lacking air shower were all retrofitted spaces. Lack of space might be an explanation for skipping the installation of an air shower.

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In our understanding, air showers are extremely helpful in keeping a low and stable level of particles contamination (https://www.alnmag.com/article/2002/12/how-do-air-showers-fit-contamination-reduction-plan). They are small (a few m²) and easy to integrate in a floor plan. Their cost is negligible (http://www.liberty-ind.com/airshower-cost.html) compared to the whole building. Moreover, they help reinforce the psychological effect that cleanliness is extremely important, while being an acceptable procedure for workers, contrary to other cleanliness measures.

Our conclusion is that air showers should be used, and strategically placed in the facility. We identified two possibilities that may coexist in the ESCF:

- Closed air shower, or closed air tunnel. This type or airlock is integrated in the workers path, to separate cleanliness levels. They can be laminar or turbulent. The latter is faster in use, and should be preferred.
- Open air showers are corridors of clean air around facility: These units operate 24 hours a day, continually scrubbing the air by recirculating it past a HEPA/ULPA filter and creating an "air curtain." The idea is that by continuously scrubbing the air, the chances of having loose particulate material in the facility is reduced. Where traditional air showers operate using high velocity air to dislodge particles, these air showers tend to work by filtering high volumes of air. In effect, you are keeping the general environment in the whole facility clean in anticipation that personnel will be bringing contaminants into the facility no matter what you do. This type of air shower works well in high traffic areas where changing garments is not practical but you still want to reduce the number of contaminants.

Cleanroom garments

Cleanroom garments are adapted to the level of cleanliness, and must be cleaned and packed accordingly. A study has been considering the effect of particles contamination reduction with usage of cleanroom garments, versus garments and undergarments. The study showed a reduction of nearly 50% in biological contaminants (skin flakes, hairs, etc.) when cleanroom style undergarments were used (Moschner 2002).

However, a complete change can be straining for the workers. It is recommended that this question should be considered depending on the nature of the samples, and on the layout of the laboratory.

3. Cleaning of cleanrooms

Based on our observations, cleanrooms for pristine extra-terrestrial samples were cleaned weekly with isopropyl alcohol (IPA)/water wipes.

A specific vacuum cleaner (e.g. http://biobubble.com/products/hepa-vacuum/) could be used, especially for changing rooms.

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VII. Transport containers for transfer of samples

In order to transfer extraterrestrial samples between ESCF's environments or from ESCF to other laboratories (and vice versa), the following requirements must be met:

- Samples kept at the same storage conditions (temperature, pressure, cleanliness, humidity).
- Minimization of forward contamination (i.e. from terrestrial environment to extraterrestrial samples).
- In case of restricted samples, no risk of backward contamination (i.e. from samples to environment/persons), since until irrefutable proof we cannot exclude that these samples include life forms which may cause permanent disability, life-threatening or fatal disease (see D6.3 for details).

1. Types of transport

Transport from landing site to ESCF

This is not discussed here as this topic was already extensively described in the D6.3 "Transport to curation facility" of the EURO-CARES project.

Transport within the ESCF

Samples should be placed in a sample container before transfer. Although the design of such a container is beyond the scope of this report, we would like to emphasize that it should be composed of at least:

- A sample collector (Figure 8, left), which should include (single) samples. The collector may also contain terrestrial material, which helps the samples manipulation, such as silicone oil or silica aerogel.
- A metallic collector protection, aimed at insulating the samples and reduce their motion. The protection should include a window or, however, a transparent material, in order to allow optical analysis of the sample. A substrate in a low-outgassing plastic material, e.g. Teflon, may be added in order to increase the insulation and/or to minimize samples contamination due to outgassing.
- A cover with latch mechanism and seals (which may be in Viton, due to its low outgassing rate).

We can then identify optional components of the sample container, depending on use, origin and storage conditions of the sample, such as:

- A pressure system, aimed at keeping the samples at the same pressure and cleanliness of storage, under an inert gas atmosphere. This would apply if the path from different ESCF environments includes critical legs, kept at different conditions of the starting environment.
- Only for restricted samples, encapsulation of the container in a sealed, low-outgassing plastic material will allow keeping people safe if container breakage would occur.

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The container does not need external protections if no critical storage conditions are required (e.g. cold temperature) and if no strong environmental (e.g. pressure, temperature, humidity) variations arise in the path between different parts/laboratories of the facility. Otherwise, the design may be more complex.

Transport from ESCF to other laboratories and vice versa

In this case the risk of both forward and backward (for restricted samples) is larger and hence the samples should be included in a more complex package, similar to that described in D6.3, i.e. a two- or three-layered package.
The requirements of the sample container strictly depend on the planned analyses on the sample. Currently, the laboratory personnel requiring the samples also provides the sample container to the Curation Facility personnel (e.g., this is the procedure used for Stardust samples). Therefore, the design of the sample container is not unique.

However, we here identify the most general requirements. As the case of transport inside the ESCF (see previous sub-section), the container should be composed of a sample collector, a collector protection and metallic walls (possibly internally Teflon-coated) aimed at insulating the samples.
When the sample is transported inside the ESCF, the pressure system (coupled with collector protection) is optional (since the internal environment is controlled) but is mandatory when the transportation occurs outside the ESCF. Figure 8 (right) shows a basic design of sample container.

The sample container would be the most internal layer of the double or triple packaging.

The additional layers aims to:

- Protect the sample(s) from forward contamination.
- Protect the container from vibrations/shocks during (ground/air) transportation.

Therefore the container should be included in a rigid and cushioned box. The box material should have low outgassing rate, in order to avoid contamination in case of container damage/breakage during the transportation. When samples need to be preserved at low temperatures, a low thermal conductivity material should be used in order to minimize heat exchange with external environment. Low temperature inside the box would be guaranteed by a cooling system, involving liquid nitrogen or a refrigeration plant. A trade-off among metallic materials is performed in the "Materials for samples containers" section.

In order to reduce forward contamination, it should be considered to fill the outer metallic layer with an inert atmosphere of nitrogen or argon gas. Trade-off analysis performed in D6.3 shows that nitrogen would be preferable due to its lower cost and to the fact that its larger reactivity and thermal conductivity are not critical for transport of extraterrestrial samples.
During the transportation phase, box pressure should be monitored in real-time: indeed, pressure change may be ascribed to box leakage or forward contamination. It would be possible to perform a further contamination evaluation after the arrival of the box to destination, by placing one or more witness plates inside of the box.

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Additional precautions must be adopted in case of transport of restricted samples, i.e.:

- The sample container should be surrounded by an absorbent material in order to prevent a risk of fluid leakage (e.g. phase transition in Martian samples).
- A layer consisting in a bag of non-outgassing plastic material must be added between the sample container and the metallic box. Whereas the double packaging (container + metallic box) reduces the risk of forward contamination, the risk of backward contamination arises for restricted samples and a safer packaging must be adopted in this case. According to World Health Organization (WHO) guidelines (WHO 2015), this triple packaging (container + plastic bag + metallic box) is mandatory for samples which may hosts life forms. It failed only in 106 cases on 4.92 million (0.002%), hence this packaging can be considered safe (WHO 2015).
- A real-time monitoring of the pressure inside the box during the transport could be needed. D6.3 shows different instrumentation/techniques that could be used to this purpose.

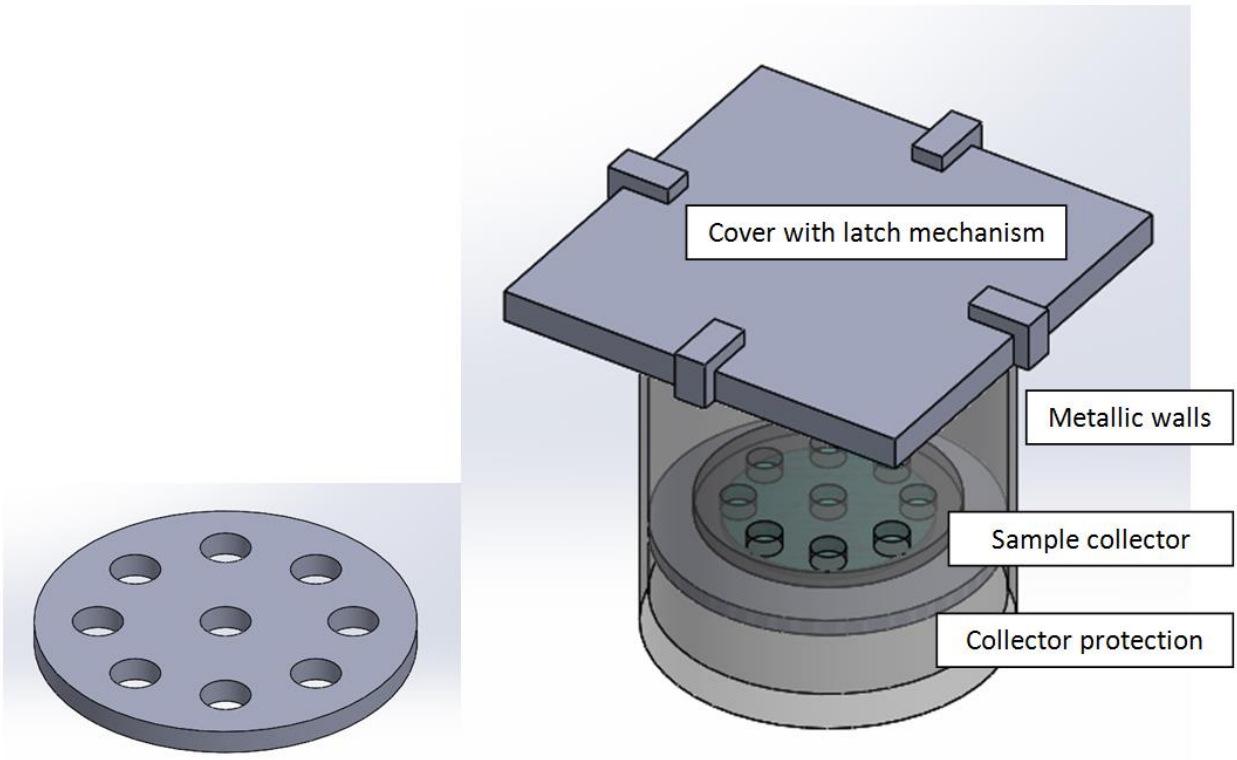

Figure 8. *Left:* Sample collector ("racket" model). *Right:* Basic design of a sample container. Depending on the study to be performed, a window of transparent material should be added in order to allow optical analyses of the sample(s).

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2. Materials for containers

Metallic materials

Metallic alloys should be preferred to other rigid materials such as carbon fiber and other carbon compounds (e.g. SiC, TiC) because of their lower outgassing rate (at least by one order of magnitude, e.g. Craig Jr 1980).

For the selection of the metallic alloys the following properties should be considered:

- *Outgassing rate.* A low outgassing rate is needed in order to minimize the risk of forward and backward contamination.
- *Rigidity and resistance to breakage.* This has to be considered only in case of transport outside the ESCF, in order to withstand to shocks (i.e. it is not a fundamental property for containers that remain inside the ESCF).
- *Thermal conductivity.* This should be taken into account in case the samples need to be maintained cold and hence a thermal insulation of the container is required.
- *Cost.* This is something to be considered only in case two materials have somewhat equivalent properties.

We do not consider density in this trade-off analysis, since the amount of samples to be transported is expected to be low (in the order of some grams) and hence containers' size is also expected to be relatively small. Therefore, container/box mass is not critical, contrarily to boxes aimed at transporting entry and return capsules (whose masses can be in the order of 10^2 kg), for which density is crucial for materials' selection (see report D6.3).
A summary of the considered properties is given in Table 5.

Alloy	**Outgassing rate (10^{-6}torr l s^{-1} cm^{-2})**	**Young's modulus (GPa)**	**Thermal conductivity (W/m . K)**	**Cost (€/kg)**
Stainless steel	0.05	195-215	16-24	1.3-1.5
Aluminum	0.6	70-80	230	1.5-1.7
Magnesium	1	40-45	120	1.6-1.8
Titanium	0.1-0.3	85-130	6	10-12
Copper	0.7	120-150	400	4-4.5

Table 5. Physical properties, thermal properties (Patrick 1973; Edelmann 1992; Koyatzu et al. 1996; Huttel 2014; Moshey 1982) and costs of metal alloys (derived from an analysis of the current market prices).

Outgassing rate and cost are the only criteria to take into consideration for transport of samples inside the ESCF, if no need of low-temperature storage. For both criteria, stainless steel is by far the most appropriate material. Young's modulus should also be taken into account for transport outside of the facility; stainless steel is again the most suitable alloy because it has the highest rigidity.
If the samples have to be kept cold, titanium may be more appropriated than stainless steel because it guarantees a better thermal insulation. However, its high outgassing rate (two orders of

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magnitude larger than stainless steel) and cost (~7 times more than stainless steel) makes stainless steel the most appropriate choice even in this case. A combination of two (or more) alloys can also be considered.

Plastic materials

According to WHO requirements, plastic material should have a good mechanical resistance and a low permeability. In addition, a low outgassing rate is a fundamental property, since it allows minimizing the risk of contamination of the samples.
D6.3 evidenced that the polymers with the lowest outgassing rate are Polyurethane (or Adiprene, polyether or polyester di-isocyanate copolymer), Teflon (tetrafluoroethylene polymer), KEL-F (or Neoflon, chlorotrifluoroethylene copolymer) and Perfluoroelastomer (or Kalrez, tetrafluoroethylene-perfluoromethylvinyl ether copolymer).
The following trade-off is therefore performed on these four materials and is based on:

- *wear/abrasion resistance.*
- *water permeability* (water resistance is a needed property).
- *nitrogen permeability* (since the box can be filled with nitrogen).
- *CO_2 permeability* (since CO_2 might be released from Martian samples and hence might contaminate the internal environment).
- *linear coefficient of thermal expansion* (it should be low in order to minimize the risk of permeability increase due to thermal expansion of the plastic material).
- *Cost.*

Table 6 summarizes these properties of the four polymers.

	Polyurethane	**Teflon**	**Neoflon**	**Kalrez**
Wear/abrasion resistance	Excellent	Excellent	Very Good	Excellent
Water permeability (10^8scmm s^{-1} cm^{-2} cm atm^{-1})	260-9500	27	0.5	40
Nitrogen permeability (10^8scmm s^{-1} cm^{-2} cm atm^{-1})	0.4-0.11	0.14	0.004-0.03	0.05-0.3
CO_2 permeability (10^8scmm s^{-1} cm^{-2} cm atm^{-1})	10-30	0.12	0.02-1	5.8-6.0
Linear coefficient of thermal expansion (10^5 °C^{-1})	3-15	5-8	4-7	23
Cost (€/kg)	0.3-0.4	5-20	20-60	3000-5000

Table 6. Properties of Polyurethane, Teflon, Neoflon and Kalrez (Peacok 1980). Peacock (1980) does not indicate the permeation data of Kalrez and the reported values are relative to Viton (having similar permeation properties). Costs have been derived from an analysis of the current market prices.

Polyurethane is not suitable due to its high values of permeability. Kalrez has overall good mechanical and thermal properties, but it is extremely costly compare to the other polymers.

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Teflon and Neoflon (KEL-F) are the best trade-off. Linear coefficient of thermal expansion are similar; Neoflon has a lower permeability to water, nitrogen and CO_2, but also a lower resistance to abrasion and is at least three times more expensive than Teflon.

We thus conclude that for the plastic bags in which the sample containers would be placed (see previous section; in case of restricted samples), Neoflon is more indicated, since samples' insulation is the most important issue, and knowing that wear and abrasion have a low probability to occur since plastic bags are expected to include small sample containers.

Otherwise, for covering the internal walls of the sample containers, Teflon would be preferred since cheaper, whereas insulation would be guaranteed by the external layers (i.e. plastic bag, if present, and the rigid box).

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VIII. Acronyms

ASRS: Automated Storage and Retrieval System

BAP: Biohazard Assessment Protocol

BSL: BioSafety Level

ERC: Earth Return Capsule

ESCF: Extra-terrestrial Sample Curation Facility

EURO-CARES: EUROpean Curation of Astromaterials Returned from Exploration of Space

FFI: Flexible film isolators

HEPA: High-Efficiency Particulate Arrestance

HSE: Health and Safety Executive

HVAC: Heating, ventilation and air conditioning

IPA: IsoPropyl Alcohol

JAXA: Japan Aerospace eXploration Agency

LPF: Laboratory Protection Factor

LD: Life Detection

LSPET: Lunar Sample Preliminary Examination Team

MSC: Microbiological Safety Cabinets

NASA: National Aeronautics and Space Administration

NIH: National Institutes of Health

PE: Preliminary Examination

PHE: Public Health England

PMSCF: Planetary Material Sample Curation Facility

PP: Planetary Protection

PPE: Personal Protective Equipment

PRF: Portable Receiving Facility

PTFE: PolyTetraFluoroEthylene

SCF: Sample Curation Facility

SRF: Sample Receiving Facility

ULPA: Ultra Low Penetration Air

WP: Work Package

WHO: World Health Organisation

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