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

STERILIZATION AND CLEANING

WP 2 D2.3

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1. Introduction

Within the next decades, the world space agencies plan to launch a variety of missions that will return samples from the surface of Mars and other celestial bodies. For example, samples from the Moon have been returned by the Apollo missions[1], particles from a comet tail by the STARDUST mission[2], as well as soil samples from a comet by the HAYABUSA mission[3], have returned samples back to Earth however; all targets so far were considered non-habitable and samples were expected not to contain any life-form. The picture is vastly different should we be able to obtain samples from Mars, a planet with the potential of harbouring life. Planning considerations must span the entire mission, including equipment and operations in space and on Earth (Race et al. 1999). Here we focus on the requirements for the return facility to:

- Reduce the probability of contamination of the sample within the facility (unrestricted and restricted missions) with microorganisms and organic material from Earth to a level as low as reasonably achievable.
- Ensure that all sample material does not contain any potentially harmful life forms or is sterilised before release from containment (restricted missions).

When returned to Earth, the first directive is to keep earth's environment and population safe, as investigated and designed by the EUROCARES project; however, great care needs also be taken that the returned samples are not contaminated by Earth-dwelling organism or material (Kminek et al. 2014). Therefore, a stringent line of methods needs to be established to protect returned samples from cross-contaminations within the facility and during handling. The following document will give detailed definitions of the terms used, an overview of currently used methods, and recommendations for the disinfection / sterilization of the facility prior to the sample arrival as well as a detailed disinfection / sterilization protocol during operations, and an outlook for further possible sterilization methods.

2. Abbreviations

CDG Chlorine dioxide gas
EO Ethylene dioxide
ERC Earth return capsule
HP Hydrogen peroxide
hPa Hectopascal
HPV Hydrogen peroxide vapour

[1] http://www.nasa.gov/mission_pages/apollo/missions/index.html
[2] http://stardust.jpl.nasa.gov/home/index.html
[3] http://www.isas.jaxa.jp/e/enterp/missions/hayabusa/

IPA	Isopropanol alcohol
kPa	Kilopascal
UV	Ultra-violet
°C	Degree centigrade
nm	Nanometer
SRF	Sample return facility
µm	Micrometer
ULPA	Ultra Low Penetration Air filter
VHP	Vaporised hydrogen peroxide

3. Definitions

Definitions listed are adapted in accordance to the European Cooperation for Space Standards (ECSS[4]).

- *Bioburden*:

  Quantity of viable microorganisms measured with a specified assay
- *Sterilization*:

  Defined as the process where all the living microorganisms, including bacterial spores are killed and the complete inactivation of enzymes. Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.
- *Disinfection:*

  The process of the elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out. Sterilization is an absolute condition while disinfection is not. The two are not synonymous.
- *Decontamination*:

  The process of removal of contaminating pathogenic microorganisms from the articles by a process of sterilization or disinfection. It is the use of physical or chemical means to remove, inactivate, or destroy living organisms on a surface so that the organisms are no longer infectious.
- *Biological ("absolute") sterility*:

  Defines the absence of all living organisms and the complete inactivation of enzymes.
- *Contaminant*:

[4] http://ecss.nl/

Any unwanted matter which could be detrimental to the required operation, reliability, or performance of a part, component, subsystem, or system.

- *Bacteriological sterility*:

  Defines the complete inactivation of all living microorganisms; however, some enzyme activity may still be recorded.
- *Practical ("commercial") sterility (Food manufacturing & conservation):*

  Inactivation of all potential pathogenic and food spoiling organisms as well as inactivation of enzymes that would compromise the product.
- *D-value:*

  Time required achieving inactivation of 90 % of a population of the test microorganisms under pre-defined (stated) conditions.)
- *Dry heat*:

  Absolute humidity of no more than 1.2 g/m^3. This is equivalent to 25 % relative humidity at 0 °C and 1000 hPa pressure or to 7 % relative humidity at 20 °C and 1000 hPa pressure.
- *Encapsulated bioburden*:

  Bioburden inside bulk non-metallic materials
- *Exposed surfaces*:

  Internal and external surfaces free for gas exchange. For example, free for gas exchange are exterior surfaces, interior surfaces of boxes with venting holes, surfaces of honeycomb cells, surfaces of the outer and inner plies of multi-layer insulation, open cell foam.
- *Mated bioburden*:

  Surfaces joined by fasteners rather than by adhesives

4. Different methods for organism removal & inactivation

This chapter will give an overview of the commonly applied sterilization/disinfection methods and a detailed explanation of the mechanisms of action of the single methods. Fig. 1 provides a glance of the later detailed methods. If available, international ISO standards[5] are given for further, detailed information's about the described process.

[5] https://www.iso.org

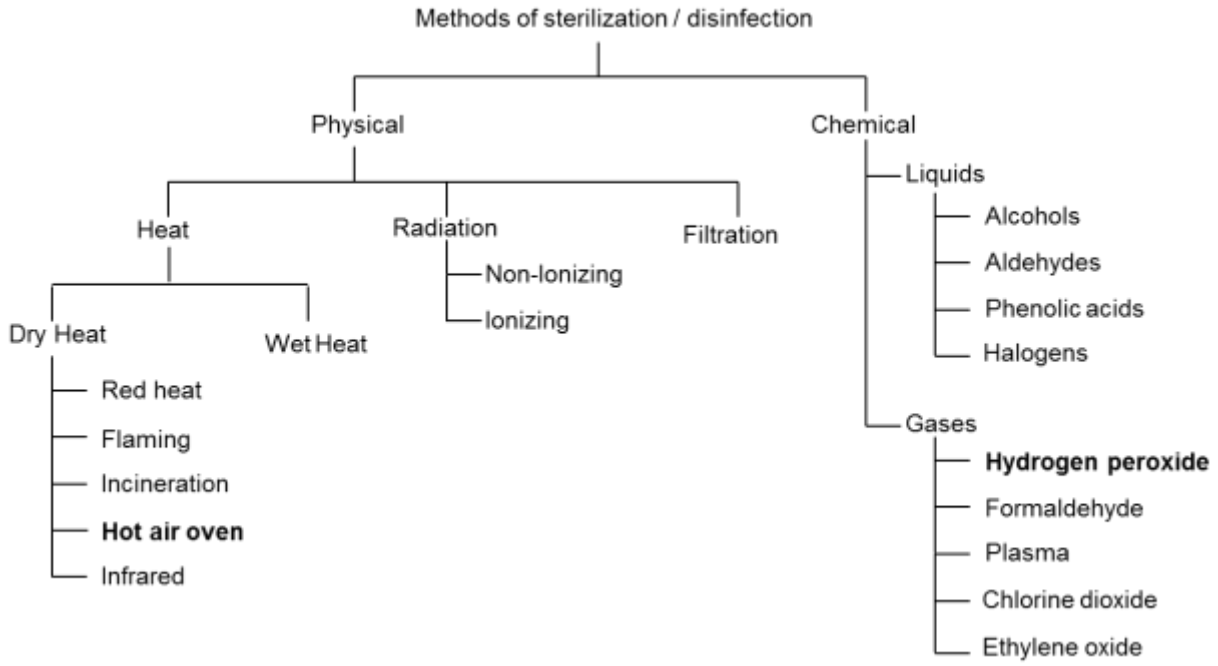

Fig 1: A schematic overview of the most commonly used sterilization / disinfection methods. Conditions highlighted in bold represent methods approved for the bioburden reduction of spacecraft and their components.

4.1 Heat

Heat is considered to be most reliable method of sterilization of articles that are thermostable. Heat acts through denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures (thermolabile) can still be sterilized at lower temperature by prolonging the duration of exposure in certain ranges. There are two different possibilities of heat exposure: **Wet heat** and **dry heat**.

a. *Wet heat:*

Wet heat acts by coagulation and denaturation of proteins. The most widely used instrument for the application of wet heat is an autoclave. Water boils at 100°C at atmospheric pressure, but if the pressure is raised, the temperature at which the water boils also increases. In an autoclave the water is boiled in a closed chamber, as the pressure rises, the boiling point of water also raises.

At a pressure of 103.4 kPa inside the autoclave, the temperature is 121°C. Exposure of articles to this temperature for 15 minutes sterilizes the majority of agents. To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135°C

or 121°C for at least one hour are recommended. Longer cycles and multiple applications can be used for dense porous materials, such as soil (Jenneman et al. 1986). The most common biological indicator for this method are spores of *Geobacillus stearothermophilus*. Further information's can be found in ISO 17665.

b. *Dry heat*:

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. The method is more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate. Such materials include glassware, powders, oils, and some oil-based injectables. Typically, *Bacillus atrophaeus* biological indicators are used to demonstrate that the dry heat sterilization process can consistently deliver the required microbial inactivation. Further details concerning this method can be found in Clark (2004), the ISO document 20857, and the ECSS-Q-ST-70-57C document.

4.2 Radiation

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate much heat, it is termed "cold sterilization". A comprehensive review and detailed explanation of this type of sterilization / decontamination method has been published by Wallhäuser 1995.

a. *Non-ionizing radiation*:

i. Microbicidal wavelength of UV radiation lie in the range of 200-280 nm, with 260 nm being most effective. It is at this wavelength that the absorption by the DNA is at its maximum, which results in the germicidal effect (Turnbull et al. 2008). UV radiation induces the formation of pyrimidine dimers as main UV photoproduct in addition to several others, which ultimately inhibits DNA replication and readily induces mutations in cells irradiated with a non-lethal dose (Sambol and Iwen, 2006, Cadet et al. 2014). Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds depending on the fluence. UV radiation is employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc. Disadvantages of using UV radiation include applicability only for surface treatment due to the low penetrative power, challenges in a reliable UV dosimetry for complex geometrical objects, limited life time and aging effects of the UV bulb, organic matter and dust prevents its reach (shading, shielding), as

well as UV radiation is harmful to skin and eyes. Some materials tend to become brownish and/or brittle due to material deterioration by the UV radiation.

b. Ionizing rays: Ionizing radiation is of two types, particulate and electromagnetic rays.
   i. **Electron beams** are particulate in nature while gamma rays are electromagnetic in nature. High-speed electrons are produced by a linear accelerator from a heated cathode. Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals. Sterilization is accomplished in few seconds. Disadvantages include poor penetrative power and requirement of sophisticated equipment. Some materials tend to become brownish and/or brittle due to material deterioration by the radiation.
   ii. **Electromagnetic rays** such as gamma rays emanate from nuclear disintegration of certain radioactive isotopes (^{60}Co, ^{137}Cs). They have more penetrative power than an electron beam but require longer time of exposure (depending on the isotope and the dose rate). These high-energy radiations damage the nucleic acids of the microorganism in addition to other cellular components. A dose of 2.5 kGy kills most bacteria, fungi, viruses and spores and is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glassware and fabrics. Disadvantages include; unlike electron beams, electromagnetic radiation can't be switched off, glassware and other materials tends to become brownish, loss of tensile strength in fabric and some plastics. Gamma irradiation impairs the flavour of certain foods. *Bacillus pumilus* E601 is used to evaluate sterilization process.

4.3 Filtration

Filtration does not kill microbes, it separates them out. Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates by special affinity, concentrating bacteria, clarifying fluids and purifying

hydrous fluid. Filtration is aided by using either positive or negative pressure using vacuum pumps.

1. Gaseous Decontamination:

Gaseous decontamination systems such as formaldehyde, hydrogen peroxide (HP), and chlorine dioxide gas (CDG) are well established in industrial, pharmaceutical, microbiological and medical settings. They are used because of their ability to decontaminate large surface areas and efficacy in complex spaces that would be otherwise difficult and time consuming to decontaminate using liquid disinfectants (Beswick et al. 2011; Rogers et al. 2005). They are also easier to validate with appropriate organisms used as biological indicators and exposed to the decontamination process on a representative surface.

a. *Formaldehyde:*

Formaldehyde has been the longest used chemical fumigant in the laboratory setting, dating back at least to the 1930's and has for many years been considered as the gold standard decontamination approach due to being; easy to use, highly efficacious, inexpensive and practical in most settings (Beswick et al. 2011; Songer et al. 1972; Campbell et al. 2012). Formaldehyde is typically delivered by heating formalin (35%-40%) with an appropriate amount of water in a thermostatically controlled unit (Beswick et al. 2011).
Despite its historical use, formaldehyde is now restricted to facilities that can be completely sealed and have some degree of ventilation control in place to minimize the risk of human exposure. This is because formaldehyde is known to be a human sensitizer and carcinogen and can leave undesirable residues if its vapour is poorly delivered or not evacuated from the treated area within a defined period of treatment (Cheney & Collins, 1995; Nelson et al., 1986). Spores of *Bacillus atrophaeus* are used as a biological indicator organism to evaluate the decontamination process. The requirements for development, validation and routine control of the decontamination process are summarized in ISO 25424.

b. *Hydrogen peroxide*:

Hydrogen peroxide (HP) works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components. Catalase, produced by aerobic organisms and facultative anaerobes that possess cytochrome systems, can protect cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen. This defense is overwhelmed by the concentrations used for disinfection (Turner, 1983; Chung et al. 2008). The effects and efficiency of HP have been

extensively studied and developed, with the vaporized hydrogen peroxide (VHP) and hydrogen peroxide vapor (HPV) the most advanced systems.
VHP systems have predominantly been used in whole building decontamination more than in healthcare settings as with HPV systems (Campbell et al. 2012). Steris VHP generators reduce the humidity within the exposure chamber prior to injection of the VHP to ensure there is no condensation on the surfaces. This allows high concentrations of VHP to be injected into the chamber compared to other HP systems (Heckert et al. 1997; Pottage et al. 2012).
HPV systems from Bioquell generate small particles (<1µm) of HPV by dropping a 30 – 35% hydrogen peroxide aqueous solution onto a heated plate creating a vapour, this is then injected into the chamber and forming microcondensation on surfaces. The relative humidity within the chamber is not altered before vapour injection (Unger-Bimczok et al. 2008; Pottage et al. 2010; Kaspari et al. 2014). A typical decontamination cycle with Bioquell systems consist of a conditioning phase, at which HPV is continuously injected/gassed (10-12g/m^3 manufacture recommended parameters) until a dew point (>70% RH) is reached and HPV condenses on surfaces; decontamination phase, where the HPV within the chamber is mixed and held, further injections can take place if an extended cycle is required; and finally the aeration phase where HPV is catalytically converted into water and oxygen using an aeration unit (Hall et al. 2007; Lemmen et al. 2015). Spores of *G. stearothermophilus* are used to evaluate the decontamination process.

c. *Chlorine dioxide*:

Chlorine dioxide gas (CDG) is a water soluble, yellow green gas. In the aqueous form it has been extensively used as a biocide in water and food treatment industries for more than two decades (Fukayama et al. 1986; Han et al. 2003; Gordon and Rosenblatt 2005). Like HP, CDG is an oxidizing agent and is reported to have 2.5 times the oxidation power of chlorine (Lorchheim 2011). Its mode of action against endospores is thought to be directly against the cell membrane rather than DNA (Young & Setlow 2003). It is perceived that the damage to the inner spore membrane by CDG causes the spore germination to halt after the initial step. CDG will primarily react with organic molecules and is generally not affected by typical organic loads as other oxidizing agents such as HP might be (Luftman et al. 2006). However, CDG is unstable, especially in the presence of UV light, and must be generated on site as needed because it cannot be produced off site and shipped (Luftman et al. 2006; Czarneski & Lorcheim 2005). Spores of *G. stearothermophilus* are used to evaluate the decontamination process.

d. Ethylene oxide (EO)

The EO high reactivity, as expressed by the high energy of its exergonic combustion reaction, in combination with its high diffusivity is of major importance for the inactivation of microorganisms (Bommer et al. 1987; Mendes et al. 2007). EO is a direct alkylating agent that does not require metabolic activation. Its microbiologic inactivation properties are considered to be the result of its powerful alkylation reaction with cellular constituents of organisms, such as nucleic acids and functional proteins, including enzymes, which leads to consequent denaturation.

The ability of a chemical to serve as an alkylating agent, and to cause mutations in a variety of biologic test systems, is widely accepted as an indicator that the chemical may have carcinogenic potential. Both alkylation and mutagenicity potential have been demonstrated for EO (Angerer et al. 1998). The validation of EO decontamination processes, which is includes physical and microbiological performances, is described in in detail in ISO 11135. *B. atrophaeus* spores are used to evaluate the decontamination process.

5. Cleanroom cleaning technologies

Cleaning can be defined as limiting contamination to a level below practical, achievable, justifiable, and verifiable limits. In 1988, NASA released a document (SN-C-0005) on the contamination control requirements of space shuttles and defined the following levels of cleanliness:

- GC (Generally Clean) - Freedom from manufacturing residue, dirt, oil, grease, processing debris or other extraneous contamination. This level can be achieved by washing, wiping, blowing, vacuuming, brushing, or rinsing. This level shall not be designated for hardware that is sensitive to contamination.
- VC (Visibly Clean) - The absence of all particulate and non-particulate matter visible to the normal unaided (except corrected vision) eye. Particulate is identified as matter of miniature size with observable length, width, and thickness. Non-particulate is a film matter without definite dimension. This level requires precision cleaning methods, but no particle count.
- VC + UV (Visibly Clean Plus Ultraviolet) - VC (visibly clean) and inspected with the aid of an ultraviolet light (black light) of 3200 to 3800 Angstroms wavelength (3.2×10^{-7} to 3.8×10^{-7} meters). This level requires precision cleaning methods, but no particle count.

Space material is routinely assembled in cleanrooms, which create an environment in which the material can be packed or worked with without the risk of microbial contamination. They can be grouped in different ISO classifications 1 to 9 which class 1 denoting the cleanest environment (ISO 14644-1:2015). A key element of every cleanroom is the air filtration system (HEPA filters), to exclude dust and particles of a predetermined size (> 0.5 µm). The most important goal of a cleanroom is to remove particles that still enter the cleanroom despite the presence of potent air filters (e.g. due to human presence). Other tasks are the removal of potential residues of previous sterilization methods (e.g. VHP) and the removal and killing of bacteria. In order to achieve these goals, cleaning is routinely performed in three steps.

1. Gross cleaning: removal of particles >50 µm
    a. Gross cleaning may be performed with special vacuum cleaners. For example the MicroVac^{Tm} vacuum cleaner incorporates a four stage filter system to remove particles and dust from the air before returning the air to the workplace. This system employs a ULPA filter, which is 99.999% effective in removing particles 0.12 µm or larger.
2. Precision cleaning step 1: removal of particles 10 – 50 µm
3. Precision cleaning step 2: removal of particle >10 µm
    a. Precision cleaning step 1 and 2 are achieved by wiping of the surface using lint free mops and wipes.

This cleaning procedure is to be completed on a regular basis, and in particular following decontamination events to remove any residue due to the decontamination routine. Following the rigorous cleaning regime described above, the selection of the right disinfectant is key to control the environment within the cleanroom. Disinfectants are a diverse group of chemicals that reduce the number of micro-organisms present within any given area. Disinfectants vary in their spectrum of activity, modes of action and efficacy. For example, some disinfectants are effective against vegetative Gram-positive and Gram-negative micro-organisms only, while some are effective against fungi. An extensive review about the different sorts of antiseptics and disinfectants has been published by McDonnell & Russell (1999). Disinfectants are employed by either spraying or wiping following the guidelines EN16615 or the ASTM standard E2967-15 (Sattar et al. 2015). For example, pre-saturated wipes with a blend of 70% Isopropyl alcohol and 30% deionized water is very effective when wet cleaning is required or after minor spills and contaminations.

6. Bioburden reduction procedures for space hardware

Space hardware is currently exposed to two approved methods of bioburden reduction: Dry heat treatment (described in detail under Point 1.b), and vapour hydrogen peroxide treatment (described in detail, under Point 4.b) following the European Cooperation for Space Standards (ECSS) detailed in the documents ECSS-Q-ST-70-56 and ECSS-Q-ST-70-57. Other methods that have previously been used are listed in Table 1.

Table 1: Main bioburden reduction methods for space equipment

Type	**Methods**	**Sterilization type**		**Heritage**	
		Surface	**Bulk**	**Studied**	**Studied and used**
CHEMICAL	Formaldehyde gas	X	--	Space components (US	--
	Ethylen oxide (EO)	X	--	--	Ranger 1961/62
	Sporicidal solution	X	--	Mars 96	Mariner Mars 1971
	Hydrogen peroxide (Vapor)	X	--	--	Mars96, Beagle2, DS2
THERMAL	Dry Heat	X	X	--	Viking, Mars96, Pathfinder, Beagle2, MER, Phoenix, MSL
STEAM	Steam (space hardware excluded)	X	--	--	Excluded on space h/w, only GSE, garments
RADIATIVE	Gamma / Beta radiations	X	X	--	Mars96, Beagle2

7. Advantages & Disadvantages of the methods

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

Table 2: Parameter weightings

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

Table 3: Scoring criteria

Score	**Residue formation**	**Material Compatibility**	**Biological efficacy**	**Reproducibility / process control**	**Cycle duration**	**Volume decontaminated by one unit**	**Cost (€) per unit (including consumables)**
3 Excellent	None, low oxidation of surfaces	Shown for all relevant materials using accepted standards	Peer reviewed scientific publications	All environmental parameters controlled & recorded	<2 hours	>100 m^3	<10K
2 Satisfactory	Limited non-organic residues	Incompatible with limited number of components	Limited peer reviewed scientific publication/ independent test reports	Some environmental parameters measured & controlled	2-6 hours	100 - 20 m^3	11 – 75K
1 Poor	Large number of residues	Little evidence/ incompatible with large range of relevant material	Company Funded test reports / advertising Claims	No environmental parameters controlled or recording process	>6 hours	<20 m^3	>75K

Table 4: Technology trade off selection

		Technology					
Parameter	**Weighting**	**VHP**	**HPV**	**EO**	**CDG**	**Formaldehyde**	**Aerosolised hydrogen peroxide**
Biological efficacy	3	9 (i.e. score 3 x weighting 3 = 9)	9	9	6	9	6
Material compatibility	3	6 (2x3=6)	6	6	3	6	3
Reproducibility/ process control	3	9	6	9	9	3	3
Residue formation (including oxidation products)	2	6	6	2	4	2	6
Cycle duration	2	6	6	2	4	2	4
Cost	1	2	2	2	1	3	2
Volume decontaminated	1	2	2	1	3	2	2
Total	(46)	**40**	**37**	**31**	**30**	**27**	**26**

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

8. Future technologies

A number of technologies have been developed and are either being used commercially in small scale or that are having research undertaken to determine their efficacy against biological agents or their practicality for use.

Methyl bromide

Methyl bromide (MeBr, also known as bromomethane) is a colourless and odourless gas. MeBr has a long history of use within the pest control (USEPA registered pesticide) and agriculture industries (Wood et al. 2016). One drawback that requires MeBr production and use to be closely sanctioned is that it is an ozone depleting substance (Serre et al. 2015). One method to reduce its impact on the environment after use is to capture the gas prior to release, this has been demonstrated using an activated carbon system that can capture more than 99% of the MeBr exhausted (Wood et al. 2015). MeBr does have a number of advantages when used as a gas for decontamination of internal spaces. It is sporicidal, so will be able to inactivate hardy bacterial spores that can commonly persist on the surfaces in cleanroom facilities (Wood et al. 2016; Sandle 2011). It can work at both low and high relative humidities, although at high humidities it is necessary to increase the MeBr concentration and temperature to maintain effectiveness (Wood et al. 2016). It is stable in gaseous form, can penetrate certain materials and is compatible with a number of surfaces (Wood et al. 2016; Serre et al. 2015). However the scientific literature on MeBr is limited and there is a lack of validated data for this technology.

Non-thermal plasma

Non-thermal plasma is a relatively recent antimicrobial process which has been tested predominantly in the laboratory and food production industry. Non-thermal plasma is a gas that is neutrally ionised, where the particles are in constant interaction with each other. The non-thermal plasma is composed of neutrons, electrons, photons and free radicals (Kyi et al. 1995). The terminology 'non-thermal' refers to the temperature that the plasma is generated at, as opposed to thermal plasmas which are generated using a high amount of energy and at high temperatures (>4000°C for arc plasmas), non-thermal plasmas are generated at a temperature close to room temperature (Tran et al. 2008).

Generally, high voltage electricity or other energy inputs are used to ionise gas molecules, thereby imparting reactive properties. The benefits of non-thermal plasma are that it is waterless, uses no disinfection chemicals and is contact free from the surface (Moreau et al. 2008). Given the reactive nature of non-thermal plasmas, they been used for surface treatment of thermolabile materials as electronics, polymers and metals (Niemira et al. 2014). But the reactive nature of the technology requires it to be generated close to the surfaces (approximately <10cm) and only small areas can be treated at any one time. Non-thermal plasmas have shown promise for the direct treatment of fresh fruits and vegetables to prolong shelf life. (Niemira et al. 2014).

In a recent paper the applicability of cold surface micro-discharge plasma for the decontamination of space hardware was demonstrated (Shimizu et al. 2014). The survivability of spores from several *Bacillus* species could be reduced by up to six orders of magnitude after a 90 min treatment. With this technique large areas can be treated by

transported chemical species almost at RT. The technique can be a potential alternative for the current dry heat method.

Non-thermal plasmas can also be used in conjunction with other antimicrobial processes, e.g. hydrogen peroxide vapour is injected into small vacuum chamber then a radio frequency signal is used to break the hydrogen peroxide molecules apart and create hydroxyl radicals (Kyi et al. 1995). This approach has been used in the medical and space industries for decontamination of equipment that can be placed within the vacuum chamber (Chung et al. 2008).

9. Recommendations

The sample return facility will include of a number of different working areas that will have different requirements for decontamination. These are detailed below:

a. Unrestricted areas in a sample return facility

Within the unrestricted sample return area of the facility there will be no requirement to use gaseous decontamination or sterilise equipment or surfaces after they have been set up. This part of the facility will not be looking at either extant or extinct life and as such will only require cleaning of surfaces to physically remove chemical contamination. Surfaces will be regularly cleaned with ultrapure water or IPA.

b. Restricted areas in a sample return facility

The restricted sample return areas will be required to be both free of biological and chemical contamination so that any analysis results will be a true reflection of what is in the returned sample. There will also be a need to ensure that any sample material leaving the facility is sterilised, if any life form is found in the sample or before it is proven that there is no life form in the sample. This is one of the reasons it is important to maintain a sterile working environment because a false positive life detection test (from terrestrial contamination) could lead to samples being withheld or an increase in expenditure from the necessity of sterilising every sample leaving the facility. Samples returned to the facility are thought to be predominantly cored rock samples or regolith. The likelihood of a solid rock containing a lifeform is very low, unless there are fissures or pores from the surface to the interior that are large enough to allow microorganism along them. The penetration of liquids or gases into these pores of into fine regolith will be low (unless there is a large pressure exerted on them for a long period of time). As such the only practical sterilisation techniques for solid or regolith returned samples will use exposure to physical energy, either from heat or radiation for sterilisation as they will be able to penetrate through them. Both will cause structural and chemical changes to the returned material that will need to be minimized and accounted for by scientists characterizing the material. Therefore the facility will require either an oven for heating samples that will be released to other laboratories for study or a gamma radiation source. If an oven is chosen then testing will need to be completed to determine the most appropriate temperature/duration cycle and sample container material that will sterilise the samples, provide the minimal alteration to their structural or chemical composition, or release off gasses from the sample container contaminating the sample material. Whilst these sterilisation methods can be used for smaller items that fit in the respective exposure chambers, they are not applicable to the interior surfaces of the SRF or for many technology items that will be used in the facility so alternatives need to be identified in addition.
During the sample receiving process a number of different sterilisation and cleaning cycles will be required. The external surfaces of each of the layers of containment for the Earth Return Capsule will need to be cleaned to remove gross debris then sterilised using either liquid or gaseous technologies to avoid transfer of Earth microorganisms and chemicals to the inner layers and sample material. Initial gross contamination removal can be achieved using ultrapure water and a number of rinsing steps, and then sterilisation can be achieved with immersion into a chemical bath, such as sodium hypochlorite. The duration the sample

container will be immersed for will be dependent on the chemical chosen and the concentration used. This process might not be applicable if there has been a non-nominal landing and containment failure because this might lead to the ingress of disinfection chemicals to contact the sample. The chemical disinfectant can then be rinsed off the Earth return capsule (ERC) using ultrapure water and this process can be repeated for each of the containment layers necessary.

There will be a requirement to sterilise the facility prior to the sample arrival to ensure there is no transfer of Earth microorganisms to the extraterrestrial samples or transfer of microorganisms between samples. For this process it would be envisaged the use of a gaseous decontamination technology. Gaseous decontamination methods allow a means to access both exposed and internal surfaces within and enclosure (such as surfaces within equipment) where liquid applications would cause issue with the technology's workings. The gaseous technologies will be able to decontaminate larger volumes and surface areas in one cycle in comparison to liquid applications that will require more person hours and may not be uniformly applied over the surfaces.

All of the available gaseous technologies will produce residues in the chambers they are used in. This will be either directly from the chemical used depositing on the surfaces or from oxide formation on the surfaces. Whilst they will inactivate the microorganisms present they will not remove them from the surfaces, which may lead to the detection of biological components from these agents e.g. amino acids or nucleic acids. Therefore it will be necessary to undertake an additional cleaning step after decontamination the remove any residues and microorganism components after decontaminant application, in addition to the cleaning step prior to decontamination. This could be completed using methods similar to those in the Johnson Space Centre Lunar facility where the interior surface of the cabinet are rinsed with molecular grade water to remove residues. It would be advisable that any identified decontamination or cleaning systems will need to be validated for use prior to selection. Testing of the surfaces after application and cleaning would help to determine the levels of remaining residues or organic contaminants prior to testing, if any are detected then this may form part of the background levels and inform the detection limits of the assays. Detailed testing of the surfaces and technologies which will be used in the SRF against the chosen decontamination regime should be completed to ensure that they are not damaged or the functionality reduced after decontaminant application (ECSS, 2008).

Of the gaseous technologies a trade-off has been completed in Table 4 to identify the most applicable for use in the SRF. The trade-off has been performed to identify the most applicable technology for the sample return facility. Several parameters have been used to and these have been weighted for their importance within the facility. Of the six technologies that have been identified vaporised hydrogen peroxide (VHP) scored the highest and therefore has been identified as the most applicable for use in the facility. VHP was closely followed by hydrogen peroxide vapour (HPV). These systems scored highly for producing the least residues, and being compatible with a large selection of material (to reduce the chance of degradation from multiple exposures). They also both have good biological efficacy against a range of microorganisms (viruses, bacteria, and fungi). This will be important as any life form returned in a sample could exhibit resistance to decontamination technologies such as some of Earth's extremophiles e.g. radiation resistance of *Deinococcus radiodurans* and chemical resistance of *Bacillus* endospores. VHP scored more highly than HPV because its

cycles are more controlled and therefore reproducible. VHP will regulate the relative humidity and volume of hydrogen peroxide that is injected into the chamber, where HPV will inject the required hydrogen peroxide depending on the conditions within the chamber. This leads to slightly different cycles each time it is used unless the conditions are exactly the same.
Overall there are a number of sterilisation technologies that are required for the SRF and whilst recommendations can be made on the choice of technologies, it will be necessary to perform a testing and validation regime to identify the most appropriate depending on the agreed parameters.

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