Trabajo Fin de Máster Máster Universitario en Ingeniería Aeronáutica

Feasibility study of storage of biological material at cryogenic temperatures in space. Part II: Experiment in the ISS

Autor: Juan Antonio Almagro Martos Tutor: Ramón de Jesús Risco Delgado

> **Dpto. Física Aplicada III Escuela Técnica Superior de Ingeniería Universidad de Sevilla**

> > Sevilla, 2018

<u> 1980 - Johann Barbara, martxa alemaniar a</u>

Trabajo Fin de Máster Máster Universitario en Ingeniería Aeronáutica

Feasibility study of storage of biological material at cryogenic temperatures in space. Part II: Experiment in the ISS

Autor: Juan Antonio Almagro Martos

Tutor: Ramón de Jesús Risco Delgado Profesor titular

Dpto. Física Aplicada III Escuela Técnica Superior de Ingeniería Universidad de Sevilla Sevilla, 2018

Abstract

This project aims to take the first steps in the feasibility study of storing cryopreserved biological samples in space. The main motivation of this idea is the economic savings that would be, in the long term, the use of a passive system in space instead of the use of liquid nitrogen on Earth, as it is done until now.

First, a brief study of the sectors where this application would be useful, the conditions to which the cryopreserved samples must be so as not to damage them, and the conditions they must face in space.

After this, a series of alternatives are proposed for possible first experiments in this area, and, in the case of this project, it is decided to carry out a first design of what would be an experiment on the International Space Station.

For this purpose, all the systems of the Station that are going to be necessary to carry out the experiment are described and, based on this, the procedure that would be followed by the mission is elaborated, according to its most important points: payload, safety, launch, installation and return.

Finally, the future lines to follow in more advanced phases of the design are presented and the potential of this research for a not too distant future is revealed.

<u> 1980 - Johann Barbara, martxa alemaniar a</u>

Contents

ix

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

The project developed below is part of a set of two documents, whose names are:

- Feasibility study of storage of biological material at cryogenic temperatures in space. Part I: Experiment in a small satellite.
- Feasibility study of storage of biological material at cryogenic temperatures in space. Part II: Experiment in the ISS.

Although this document corresponds to the second part of the project, it is not necessary to have knowledge of the precedent to understand it in any case.

The authors of both documents have proposed it so that both depart of a common preliminary study, which, as can be seen in the subsequent development, reaches a point that gives room to more than one line of research. It is at that point where each of the ideas is developed separately and independently in each of the memories.

The sector that is spoken of in this project is still unknown, and, as will be described below, there are organizations that are already showing interest in it, there are still no investigations or developments in this area.

For this reason, it has been necessary for the development of the project to drink from different sources of information, some of them bibliographic and based on the written procedures developed for each particular system, but it has also maintained contact with professionals in the sector, which have been helpful at certain points of development.

Specifically, the authors of both projects have been in close contact with Chris Barber, founder of the ISSET organization, which promotes the scientific dissemination of space issues.

To this end, the authors were invited to participate as mentors in Mission Discovery programme, organized by this organization, and whose purpose is the selection of a space project developed by teenagers to carry it out on the International Space Station.

During the event, there was the possibility of exchanging knowledge about the idea of the project, both with Mr. Barber and with other professionals, such as NASA astronaut Michael Foale.

2 STATE OF THE ART

2.1. Cryopreservation

Cryopreservation arises from the need to maintain cellular viability and functionality for long periods of time. For this, it is necessary to stop any biological activity that takes place in the cell or tissue, for example, the biochemical reactions that could cause cell death. This is achieved by subjecting the samples to a cooling process in which they are frozen at very low temperatures, usually between–80 \degree C and −196 \degree C.

The methods used in the cryopreservation process are called protocols, which, depending on the rate of cooling and subsequent heating, can be classified into:

- Slow freezing slow thawing.
- Slow freezing fast thawing.
- Ultrafast freezing.
- Vitrification.

Slow freezing is characterised in that the cryoprotectant is added step by step and the rate of temperature decrease is controlled using a programmable freezer. Ultrafast freezing and vitrification consist of dehydrating the cells with the use of high concentrations of cryoprotectant and, subsequently, immersing the samples in liquid nitrogen for cooling, which means that the use of programmable freezers is not necessary. On the other hand, the rapid thawing process can be carried out at room temperature or by performing a water bath at 30° C. Likewise, during the period of time that the samples remain frozen these are usually stored in freezers of -80° or in tanks of liquid nitrogen that keeps them at -196° C, the latter being the most usual method.

The origins of cryopreservation date back to 1946 when Rostand focused his studies on the freezing of gametes from amphibians. Three years later, Polge would focus these studies on birds and, later, on mammals. In fact, in 1952, Polge and Row cryopreserved bull sperm for the first time, obtaining very good results.

In the 1960s, there was a large demographic expansion in the United States, which required increasing the amount of beef, as well as the production of milk. In order to reduce costs it was decided to resort to artificial insemination, which led to great advances in the methodology used in the cryopreservation of sperm.

In the 1970s, a step was taken and numerous embryo cryopreservation studies were carried out using mice, rabbits and sheep. In 1979, Trouson and Mohr cryopreserved a human embryo, however, despite the fact that after implantation the pregnancy started, it ceased after 24 weeks. Finally, in 1986, Testart and Lasalle published the first successful pregnancies of frozen embryos.

Currently, the cryopreservation of gametes and embryos has protocols that are sufficiently standardised and the process has a high success rate. In addition, it is a technique that is socially accepted, thus differing from cryonics, cryopreservation of the human being.

2.1.1 Cryonics

Cryonics consists in the cryopreservation of people once they have been declared dead, with the aim of returning them to life in a future in which science has found a cure for the disease they suffered.

The first reference that was made about the possibility of keeping human life in standby for long periods of time is 1773 and it has been collected in a letter written by the American Benjamin Franklin. However, the most similar concept to the current cryonics was proposed by Robert Ettinger, in 1962, in his book *The Prospect of Immortality*, where he pointed out that the freezing of people would favour the development of more modern medical technologies. In 1965 the German Karl Werner coined the cryonics word for the first time, while the creation of several foundations began, being the first one the Cryonics Society of New York foundation.

Throughout history, cryonics has suffered numerous ups and downs due to being subjected to a constant analysis of the legal, moral and ethical aspects that concern it. Cryonics is based on the fact that both memory and personality remain stored in the structure and brain chemistry, a fact that is also accepted in medicine. Likewise, it has been shown that brain activity can be reactivated, under certain circumstances, after having stopped. However, the reversibility of neuropreservation is still, to this day, a fact that generates controversy of opinions. Unlike humans or large animals in which cryopreservation is still irreversible, small organisms such as *Caenorhabditis elegans* have been successfully cryopreserved and, once thawed, it has been shown that their memory remained intact.

At present, cryonics is a practice that is legalised in very few countries, with the USA and Russia hosting the most leading companies in this sector, Alcor and KrioRus, respectively. Each company offers different cryopreservation services to its clients, highlighting DNA cryopreservation, neuronal cryopreservation and cryopreservation of the whole body, KrioRus also offers the possibility of cryopreservation of pets.

The Life Extension Society Foundation (now the Alcor Life Extension Foundation) was the pioneer in the cryopreservation of people, with James Bedford being the first human being to be cryopreserved in 1967. It is currently estimated that there are a total of 300 people in the world who have been cryogenised, with hope that in the future there will be the means and technology that will allow them to be brought back to life.

2.1.2 Storage centres for genetic material

Given the increasing number of species on the verge of extinction that exist on the planet, projects have emerged consisting of the creation of storage banks for genetic material from endangered or extinct species.

With this DNA and live cryopreserved cells the expectation is to be able, some day, with the sufficiently mature cloning technology, to resuscitate extinct species. Meanwhile, it is a genetic library of incalculable value.

An example of this type of project is the one known as The Frozen Ark Project, carried out jointly by the Zoological Society of London, the Natural History Museum, and the University of Nottingham. This project takes samples of animals in zoos and those on the path to extinction in the wild.

At the national level, in Spain, there are also similar projects, such as the BanGES (Bank of Germplasm), established in 2003 by the CSIC and the Ministry of the Environment, which develops research and conservation of threatened species in the peninsula. The biological material that stores is the germplasm (sperm, ovules and embryos), in addition to tissues and somatic cells (those that make up the growth of tissues and organs). The main objective, in the same way as the rest of similar initiatives, is to use this material for reproductive and genetic characterisation.

2.2. Temperature conditions for cryopreservation

Usually, temperatures of around −80℃ are used to keep cryopreserved organisms. However, this temperature is not stable for long-term storage of the samples, these being perishable.

This is because at −80°C there is an ice growth that with long-term storage can lead to excessive growth that damages organisms, especially if they are large.

To carry out a long-term storage, which may become undefined, the storage temperature must be the glass transition temperature, T_g . At this temperature, the fluid undergoes a second-order phase transition, in which it passes from a viscous fluid to a solid, increasing its viscosity by approximately 15 orders of magnitude.

The glass transition temperature in case of typical solutions used for cryopreservation is about −140°C, so this temperature is considered optimal for long-term storage. Storage at these temperatures is called "storage at intermediate temperatures", since the triple point of nitrogen is −196℃.

Storage at lower temperatures of −140℃ is not recommended, since mechanical stress leads to the growth of the fractures, without having a biological advantage for the cryopreserved samples. If it is desired to lower the temperature, it could be done in such a way that the transition is very slow, which causes the release of mechanical stress and the fractures do not grow, however, this process is very complex.

For long-term storage, temperatures below −140℃ produced by the temperature cycles are not desirable, since the phase change occurs and the fractures grow again. Due to this fact, it can be established that an ideal window for the maintenance of the samples would be between -140° C and -150° C.

The existence of a thermal cycle is not excessively harmful if it is small samples, since the appearance of fractures would not imply the breaking of them, even so, it is not desirable to have a very large number of cycles to damage to a lesser extent. Possible cryopreserved organisms.

The use of a dynamic system for temperature control is not highly recommended for the control of stored samples, since temperature control systems are often subject to many faults. The use of a passive system is recommended.

2.3. Cost of liquid nitrogen

In this section, we will estimate the economic cost of maintaining cryopreserved organisms on Earth, using liquid nitrogen tanks.

The main expense is the replacement of the loss of nitrogen by evaporation and leaks in the containers. In this study, only this fact will be taken into account and other expenses will not be considered, such as the cost of the personnel that supervises and fills the tanks continuously and the facilities and infrastructure necessary for the proper conservation of the organisms.

First, an estimation of the centres that use liquid nitrogen tanks will be made, then a calculation of the approximate nitrogen losses of the same and, finally, with the price of nitrogen, the costs will be calculated.

2.3.1 Centres that use liquid nitrogen in medical sector

Only those centres dedicated to the medical sector, which are of interest in the study carried out in this project, will be taken into account.

In addition to these centres, the industrial sector also uses liquid nitrogen for certain applications, such as the shrinking of the screws during the assembly phase of the structures. However, they will not be considered since the context in which they are used is completely different.

For the example that has been exposed, a quick arrangement of the pieces is needed for their assembly. In addition, storage times at very low temperatures are usually very low, so the space application is discarded.

The estimation of the number of centres will be carried out at a national level, in Spain, except for cryonics centres, for which the two most important companies in the world will be taken into account.

The sectors that have been taken into account are In Vitro Fertilisation centres (IVF) and organ banks. For each of them, the approximate number of tanks used and the capacity of them according to the organisms with which they work are estimated:

- In Vitro Fertilisation Centres (IVF):
	- o Around 300 centres in Spain.
	- o Each centre has 5 tanks of about 30 litres.
- Organ Bank:
	- o It is going to estimate that there is about one centre per province capital, so around 40 centres in Spain are calculated.
	- o Each centre has 10 tanks of about 50 litres.
- Cryonics:
	- o Two important companies worldwide, *Alcor* and *Cryonics Institute*.
	- o Each one has 100 tanks of about 2000 litres.

In this study, other types of institutions such as biotechnological research centres or repositories (biobanks that exclude tissues) have not been considered. In these cases the consumption is lower, and the dispersion much higher. In addition, these centres are not dedicated to storage, so they are not so representative of the focus of this project. In the same way, there are more cryonics centres around the world, of which there are not as many

data as the two presented. These facts mean that the cost is also underestimated, adding the above in the introduction of the section.

Given that the estimation of the institutions has been carried out at the national level and that there are more cryonics centres, in order to extrapolate the results obtained at an overall global cost, it has been decided to multiply by a factor of 10 the results that will be obtained.

2.3.2 Losses of liquid nitrogen

In this section, the losses of liquid nitrogen that are produced by evaporation through the containers will be established to estimate the daily amount of nitrogen that must be supplied to keep them full.

To carry out this study, we have consulted the catalogues of Cryo Diffusion company, one of the most important suppliers of cryogenic storage tanks in the world. All models, both small and large capacity, have been taken into account.

For each of them, an adjustment has been obtained that aims to be no more than an approximation of the relationship between the capacity of the tanks and their losses, so that all models are taken into account.

The study was carried out separately for large and small tanks, so it is possible to approximate a little better each one of the two cases, since, as explained in the previous section, the size of these will be significantly different according to the type of centre.

2.3.2.1 Large tanks

In the case of large containers, the models shown in Table 1 have been collected. In it, those models that appear with asterisks are focused on the industrial sector, with a higher handling rate due to the nature of their use. Therefore, although they have been collected in the table to show the entire catalogue, they will not be taken into account when making the adjustment.

Using the data shown, the adjustment corresponding to this type of pitchers has been made, shown in Figure 1.

Various types of adjustment have been contemplated for the fulfilment of the adjustment, however, according to the trend that follows, it has been decided to perform a logarithmic adjustment. Even so, the calculation of the coefficient of determination has been made for each type of adjustment to check if it is the one that best fits the data. Results are shown in Table 2.

It is verified, therefore, that the best fit is the logarithmic, as predicted.

Large cryopreservation tanks

Figure 1. Large cryopreservation tanks and adjustement

Model	Capacity (L)	Losses (L/d)	
BF-2110M	110	3	
BF-23050M	378	7	
BF-2350PM	373	7	
BF-2600M	680	10	
BF-2600PM	672	10	
BR-2048M	48,5	0,27	
BR-2100M	100	0,65	
BR-2150M	148	0,65	
BR-2200M	197	0,65	
CF-170M	188	4,7	
CF-230M	289	6,3	
CF-320M	370	6,5	
CF-350M	444	6,8	
CF-400PM	400	9	
LO-2075M	74	2,5	
LO-2200M	180	5	
LO-2250M	218	5	
SD-600M	716	6	
SD-1000M	928	8,5	
SD-1200M	1127	9	
SD-1500M	1513	9	
SD-1850M	1724	10	
$CD-45M*$	37	4,5	
$CD-60M*$	73	$\overline{4}$	
$CD-90M*$	92	$\overline{4}$	

Table 1. Models of large cryopreservation tanks

Table 2. Coefficient of determination for each type of adjustment

2.3.2.2 Small tanks

For the case of small vessels, Cryo Diffusion has two different types available, some for more continuous handling and others for long-term storage. The rate of nitrogen loss varies from one to the other, so all the models of the two types have been taken into account in order to estimate an average evaporation rate when making the adjustment, since in the centres that have had account may use both types of tanks.

In this way, all the models of small tanks of the company are included in Table 3.

Using the data shown, the adjustment corresponding to this type of tanks has been made, shown in Figure 2.

Table 3. Models of small cryopreservation tanks

9

Figure 2. Small cryopreservation tanks and adjustment

2.3.3 Price estimation

To estimate costs, it is essential to know the price of liquid nitrogen. For this, the main Spanish suppliers of these have been located, which are the companies *Airliquide* and *Carburos Metálicos*.

The prices established by the first will be used as a base, however, the price varies according to the amount of nitrogen required, being lower in the case of needing more quantity. Nevertheless, it ranges between 2.5 and 3.5 euros per litre of liquid nitrogen.

Since there are centres of such different natures, two cases can be distinguished within the typology studied. In smaller centres, which use smaller capacity tanks, a price of 3.5 ϵ /L will be established, as they will require a smaller amount of nitrogen to fill them.

On the other hand, in larger centres, in this case, cryonics products, which require large amounts of nitrogen due to the large capacity of storage tanks, will be considered a price of 2.5 ϵ /L. In this way, economic expenditure can be better estimated than using an average cost for all types of centres.

2.3.4 Cost calculation

Using all the above, it is possible to calculate the annual cost of maintaining the quantities of nitrogen in the tanks. As already indicated, this is going to be one of the biggest costs, but not the only one, so the results obtained here are underestimated.

In the same way, to extrapolate the considerations made at the national level, a multiplier of 10 will be used to calculate the annual global cost of liquid nitrogen losses in cryopreservation on Earth.

For In Vitro Fertilisation centres and organ banks, where using small vessels, the approximate losses of nitrogen have been calculated using the model of small tanks presented previously. On the other hand, for cryonics centres, which have more capacity containers, the model of large tanks has been used to calculate the nitrogen losses produced. The results are shown in Table 4, presented below.

It can be seen how the global cost of maintaining cryopreserved organisms on Earth is very high, even though costs have been underestimated. Also, note that the most important expense occurs in cryonics centres, which are those that perform longer storage.

	IVF	Organ banks	Cryonics	
Number of centres	300	40	$\overline{2}$	
Tanks/centre	5	10	100	
Total of tanks	1500	400	200	
Capacity (L)	30	50	2000	
Losses/Tank (L/day)	0.215	0.245	11.20	
Total of losses (L/day)	322.96	97.89	2240.66	
Cost of nitrogen (E/L)	3.5	3.5	2.5	
Daily cost (ϵ)	1,130.37	342.63	5,601.65	
Annual cost (ϵ)	412,585.81	125,058.32	2,044,600.88	
Annual global cost (ϵ)	41,258,581	12,505,832	204,460,088	

Table 4. Costs associated with losses of liquid nitrogen

2.4. Space environment

The accomplishment of missions in the space supposes a change of paradigm with respect to the conditions that would have to conduct them in Earth. Intrinsically, space missions have strong restrictions, for example, in:

- Weight and size: Associated with the current capacity of the launcher.
- Consumption: The power supply is not unlimited, although it is true that it is possible to use solar energy (although not in all applications). Even so, the energy consumed must be limited to the generation capacities.
- Reliability: Because of the high cost of missions, at the level of design, materials, manufacturing and launching, or because of their nature (such as manned missions), reliability must be a key factor in the design.

In addition to these restrictions, associated with the conception of the mission, in each of the phases of the mission, the characteristics of the environment vary considerably.

2.4.1 Pre-launch phase

Some considerations that must be taken into account in this phase are the following:

- The duration of the preparation of the mission, including its definition, design, manufacture and assembly of components and systems, is high. The average duration of this phase is usually between 5 and 10 years. In addition, the possible launch windows, which, according to the mission, can be very small, should also be taken into account.
- Design according to the availability of components, their cost and certification of their quality.
- Given the high reliability required, as already mentioned, the performance of certain tests or assemblies requires a very controlled environment, for example, in temperature, pressure, humidity or dirt. It is by this factor also that the fulfilment of redundant systems is required, especially of the most critical systems. There are two main categories of redundancy:
- o Active: all redundant systems work simultaneously, so they do not require external failure detection elements.
- o Stand-by: redundant systems are activated if the active fails, so external failure detection elements are required.

2.4.2 Launch phase

During the launch, there are effects that must be taken into account in the conditioning of the vehicles or satellites where the components of the mission are located.

- Vibrations: during the launch, the appearance of vibrations transmitted from the launcher's motors is inevitable.
- Shocks: related in part with the previous aspect, but also, for example, by the coupling / uncoupling performed during this phase.
- Variations of pressure and temperature: from the conditions at sea level to those of outer space, passing through the different layers of the atmosphere.

2.4.3 Operational phase

During the fulfilment of the mission the environment will also present certain characteristics that, although they vary according to the characteristics of the mission, can be generalized in the following phenomena:

- Radiation: Exposure to radiation, much more intense than on Earth, can have adverse effects on equipment and systems. In particular, some of the elements most sensitive to radiation are:
	- o Circuits, processors, wiring and insulators.
	- o LED, laser diodes, fibre optic, optical materials.
	- o Sensors and detectors.
	- o Cryogenics.
	- o Biological material.

To mitigate the effects of radiation in space it is possible to use protective shields or surface treatments, design the satellite to have an adequate placement of the instruments, or make use of components hardened to radiation.

- Temperature: the temperature ranges to which the systems in space are subjected are very extensive, from temperatures close to absolute zero, to temperatures of hundreds of degrees in direct contact with solar radiation. The sources of heat can be of different natures:
	- o Direct solar radiation.
	- o Solar radiation reflected by other elements.
	- o Planetary radiation, which is the thermal energy radiated by the planets.
	- o Radiation of the components themselves into space.

To control the temperature can be an active control, such as heaters or refrigerators, or passive, such as the use of surface finishes or specific treatments on surfaces. The first ones have the disadvantage that they consume energy resources and their installation and operation is more complex.

- Electromagnetic problems: In the platforms formed by multiple instruments it is necessary to take into account the electromagnetic interference that may occur, for which reason a design must be carried out that contemplates the compatibility of all of them, by means of isolation, screening or "grounding".
- Vibrations: As in the launch, the systems can be subject to vibrations produced by the operation of equipment. The most fragile elements to this type of phenomenon are:
	- o Printed circuits.
	- o Optical and electronic devices.
	- o Mechanisms and mechanical interfaces between pieces.

 Space debris: Any artificial object without utility that orbits the Earth is called space debris. Despite the small size of most of the fragments, the dizzying speeds at which they are subjected, make these a serious threat to any mission. It is estimated that there are more than 50000 objects larger than one centimetre, observing in Figure 3 the distribution of them by altitude. It is observed how the highest concentration ranges are those of the most used orbits for the positioning of satellites, such as low orbits or geostationary orbits.

Figure 3. Density of space debris by altitude, according to ESA

3.1 Motivation

Cryopreservation is a technique that is commonly used with different objectives, as explained above. The possibility of carrying it out in space would open up doors to a whole new field of research that, without a doubt, would be a great benefit in many areas of science and technology.

In the first place, it could mean a reduction in the maintenance costs of cryopreserved organisms, especially in the long term. This is so given that the costs associated with the process would be the placing in orbit and reentry to Earth, avoiding the costs for maintenance and replacement of liquid nitrogen during the entire duration of the process.

This fact would be really important, for example, in cryonics companies (such as those described above), which have shown a real interest in carrying out this type of mission. The maintenance costs in these centres are especially critical, since the tanks used, given the nature of the cryopreserved organisms, are large volume, which implies higher losses of liquid nitrogen and a greater expense in the maintenance of the systems.

On the other hand, and as detailed, there are projects of cryopreservation of genetic material of endangered species. These storage banks contain valuable genetic information. Since the required storage space is much lower than the previous case, the costs of liquid nitrogen are significantly lower, however, the stored material is especially critical, and a malfunction of the system, either by a failure in them, a natural disaster or an attack, would make it lose a probably unrecoverable resource.

That is why being able to establish storage space, in addition to reducing costs, would allow having a security reserve of especially sensitive material, safe from the inclemencies that could occur on the planet.

Apart from the clear benefit in the storage of cryopreserved material, the study of space cryopreservation would open another way to the possibility of long-lasting manned space travel, enabling the cryogenization of the crew without the need for expensive and sensitive equipment during the entire travel time. It would be enough with a system of initiation of the cryopreservation (with the adequate procedure not to damage the person) and another system to reheat it at the end of the journey.

It is verified how space cryopreservation is a technique that, if possible, would offer enormous economic and technical benefits in many fields of science and research.

3.2 Objectives

Space cryopreservation is an unprecedented method that has never been attempted before. However, all the advantages that its use would imply if it turns out to be feasible have already been indicated.

That is why the ultimate goal would be to demonstrate that it is feasible to carry out space cryogenization, so that it can, for example, lower costs with respect to carrying it out on Earth, or the function of warehouses can be carried out of genetic material in space, with the advantages that have already been exposed.

Given the limitations of researching a field hitherto unknown, the scope of this work must be bounded, and the objectives to be achieved are the following:

- Propose and study different possible options to carry out cryogenization in space. Determine the advantages, disadvantages and limitations of each of them.
- Determine which is the most appropriate to perform a first experiment that demonstrates the technical and economic feasibility of space cryopreservation, according to the previous study.
- Mark the first steps to follow to be able to carry out the design of the mission.

In the development of the project, it is proposed that the biological material to be stored in the space be genetic material of species belonging to both animal and plant species, in case it is viable to conserve it, to propose in the future the space cryopreservation of already extinct species and of those that are in danger of extinction. It is decided to use this type of samples since, beforehand, the result and viability of the experiment are unknown, and in this way it is avoided to risk genetic material of protected species.

4 EVALUATION OF ALTERNATIVES

In this section, the different available options for experiments in space, specifically, cryopreservation experiments, will be presented. Subsequently, a small comparison will be made between the different options presented and one of them will be chosen for further development, which will be the core of this project.

4.1 International Space Station

The first of the options proposed for experiments in space is the International Space Station (ISS).

The ISS is a research centre and laboratory of interpretation in permanent orbit in space, in which up to seven space agencies from different countries participate.

Its orbit around the Earth is about 400 kilometres above sea level. Destined to be a laboratory, an observatory and a factory in low Earth orbit, and also act as a test base for possible future missions to the Moon, Mars and asteroids, it has funding to maintain its operation, for the time being, until the year 2024.

The ISS provides a platform to carry out scientific research that could not be done in any other way. Although other platforms could carry out experiments in a space or microgravity environment, only in the ISS there is a permanent crew and continuous transport to and from the Earth that allows us to expand the typology of possible experiments.

4.1.1 Laboratory at cryogenic temperatures

The ISS has a module that allows carrying out experiments that require cryogenic temperatures, up to −99℃. This module is called MELFI (Minus Eighty-Degree Laboratory Freezer for ISS). It is based on the reversible thermodynamic cycle of Brayton, which uses nitrogen in a gaseous state as a working fluid. This system was chosen for its energy efficiency at the desired operating temperatures and for its low disturbance of the environment on board the ISS.

This system is technically capable of operating at any set point between 10°C y −99°C, but there are three standard operating modes: −95°C, −35°C and 2°C. The temperature is continuously monitored and recorded in real time. During the shutdown phases, temperatures are still recorded. To ensure insulation between the rooms, in the space between them, double-walled, a very high molecular vacuum is pumped.

In this way, it is verified how the ISS is prepared to carry out missions and experiments of all kinds, and in particular experiments with cryopreserved material, making it a viable option to carry out feasibility studies focused on it.

4.1.2 Transport to the ISS at cryogenic termperatures

Another question that arises when considering the fulfilment of a mission in the ISS is the form of transportation to it. In the case of the Space Station, this phase of the mission is much simpler, since there are ships that continuously transport elements to and from it, both manned and unmanned.

Therefore, this phase is carried out according to the procedures of each of the ships that carry out the flight. The only thing that should be considered is the condition that the material must be stored at cryogenic temperatures throughout the journey. It should be noted that some transport vehicles to the ISS can be equipped with a module that allows the transport to cryogenic temperatures (−80°C) to and from the station. This module is known as *Polar*, and it works with a power of 75 W, using air cooling as a method of heat rejection. It can accommodate up to 12.75 L of sample volume and up to about 9 kg, including the support equipment of the same.

4.2 Small satellite

Another possible option to carry out experiments in space is the use of satellites in orbit. The artificial satellites are sent to space in launch vehicles and, once decoupled, they remain orbiting around natural satellites, asteroids or planets. Once the mission for which they have been designed is completed, they may orbit as space debris or may disintegrate upon reentry into the Earth's atmosphere.

Each satellite is designed based on the objective or mission that you want to carry out. We distinguish between: communication satellites, meteorological satellites, navigation satellites, astronomical satellites, etc. Specifically, in the case at hand, the design mission of the satellite is scientific, so it could be included within the so-called biosatellites.

The fact of proposing to carry out a cryopreservation process aboard a small satellite orbiting around the Earth is due to the peculiarity that in space the temperatures reached in the satellite zones where solar radiation does not affect are extremely low, that is, cryogenic temperatures are reached.

However, in order to optimize the viability of the samples, it will be necessary to evaluate at what point of the Solar System is optimal to put the satellite in orbit in order to minimize possible temperature fluctuations caused by the movement of the satellite in the course of its orbit. At this point, it would be a necessary condition to apply thermal protection shields that reduce the remnants of solar radiation that may affect its surface.

4.3 Moon

Another option for the storage of cryopreserved material is to place it in craters on the lunar surface. This idea has been proposed to the authors of both projects by Michael Foale, a NASA astronaut who has participated in six space missions throughout his career.

The proposal consists in placing a storage place in the depths of a place located on the dark side of the Moon, since the temperatures at these points are low enough to allow the cryopreserved material to be maintained, protected in addition to solar radiation.

The idea of keeping a certain material on the lunar surface at cryogenic temperatures has already emerged. For example, in 1965, four years before the Apollo 11 mission, Arthur D. Little made a study of the possibility of cryogenising on the lunar surface.

The main objective of this study was to develop a predictive method of thermal conditions of a container with cryogenic content during exposure to the lunar environment for a prolonged period, and analyses the losses that would occur by vaporisation.

The results would be used to carry liquid hydrogen deposits as fuel for the vehicles used in the first manned mission to the Moon, of vital importance since one of the astronauts' main tasks upon arrival would be an intensive exploration of their surface.

Returning to the idea of cryopreservation on the Moon, this can be supported by the data collected by the *Lunar Reconnaissance Orbiter* mission, a probe destined for the exploration of the Moon, launched in 2009 and still operational today, located in an orbit polar. Specifically, the instrument aboard this probe known as *Diviner* or LRO (Lunar Radiometre Experiment), consisting of an infrared radiometre, is responsible for making temperature maps of the lunar surface, with the aim, among others, of detecting deposits of ice and characterise the composition of the surface.

This instrument has reached temperatures of up to 35 K (-238° C) in some craters located in the permanent shadow zone, being one of the lowest temperatures registered in the Solar System so far (below even the registered on Pluto). In Figure 4 one of these temperature maps obtained by the instrument can be observed.

Figure 4. Temperature map obtained by the LRO instrument

In the figure it is possible to see the wide range of temperatures that are reached in different areas of the lunar surface, being able to locate the place of storage in the region with the optimum temperature range for conservation at cryogenic temperatures.

This option would be even more feasible after the construction of the Lunar Space Station, planned by NASA, and that would allow a safe and regular transport if necessary.

4.4 Options comparison and experiment choice

Different alternatives for the storage of cryopreserved material in space have been exposed, proving that all of them would be valid for the objective pursued. However, for the fulfilment of a first feasibility study it will be necessary to take into account all aspects, advantages and disadvantages, offered by each of the options and reach a point of convergence in terms of technical and economic viability.

First, the accomplishment of the experiment in the ISS would be very enclosed, since there are numerous procedures tested for sending and returning ships, handling cryopreserved material and carrying out experiments safely in general. However, the conditions in which the ship is located (orbit, available modules ...) mean that the experiment cannot be carried out under optimal conditions, since it may be the case that it is not in a permanent shadow zone. However, there is currently enough technology to mitigate these adverse effects. The fact of having knowledge of the procedures and that technologically the necessary modules already exist make this option one of the most economical, insofar as, beforehand, the estimation of the costs of the same is sufficiently bounded.

Secondly, the launching of a small satellite is the most versatile option of the three proposals given that there is greater freedom in the orbit selection, the instrumentation on board and the conditions of protection against solar radiation of the payload. From the point of view of lowering the cost of launching it would be appropriate to do it together with other satellites in the same shuttle. Nevertheless, with the technology currently available, the process of return of the samples to the Earth supposes a great complexity of the design of the mission and, consequently, it will suppose a greater cost than doing it from the ISS.

Third, the possibility of storing the material on the Moon is considered, which has been proven to be an option that allows, in addition to protecting the samples of solar radiation, to choose an optimal temperature range. Since the experiment does not require a permanent source of energy, except for some monitoring instrument that could be powered by a battery, the fact of not receiving sunlight would not be an inconvenience, but quite the opposite. However, this option is associated with the problem that, currently, transporting the samples to

the Moon is a mission that must either be manned for the subsequent handling of the cryopreserved material, or it must have a very sophisticated technology, and less safe. However, this would no longer be a problem when the Lunar Space Station that NASA wants to develop in the next few years is established.

At this point, it is necessary to evaluate the options and select one of them. For this, in Table 5, the different options developed are presented schematically, evaluating the different requirements of the experiment:

- Complexity: Measurement of the technical difficulties involved in conducting the experiment, based on its design, transportation, handling, etc.
- Cost: Economic cost that involves conducting the entire experiment, based, among other factors, for example, the fact that it is possible to do it in parallel with other experiments (joint launch, use of existing facilities, etc.).
- Safety: A factor that reflects whether the experiment is based on existing and proven procedures, or it is necessary to create new systems and procedures for its fulfilment.
- Completion: Reflects if the experiment is capable of being carried out completely, with the current technology, carrying it out through each of the options.

Option	Complexity	Cost	Safety	Completion
Satellite	Medium	Medium	Medium	No
ISS	Low	Low	High	Yes
Moon	High	High	Medium	Yes

Table 5. Comparison of the different options for conducting the experiment

In view of the comparison made, it is clear that the most viable options to carry out an experiment of cryopreserved samples are:

- ISS: It offers the possibility of carrying out the experiment in a shorter timeframe and allows the completion of the experiment with the technology currently available.
- Satellite: On the other hand, it offers the advantage of extending the useful life of the mission since it would not be subject to the needs of organizations with presence in the ISS.

It is at this point when it is concluded that it is necessary to open two different lines of research. That is, propose a project that details what would be the procedure to follow if the experiment was carried out in the ISS and another in which the storage support of the samples is a small satellite.

This project, *Feasibility study of storage of biological material at cryogenic temperatures in space*, is divided into two parts:

- Part I: Experiment in a small satellite.
- Part II: Experimento in the ISS.

In the present document, the option that will be studied in greater depth is that of carrying out the experiment in the ISS.

As mentioned above, the International Space Station (ISS) allows the accomplishment of missions in very different fields. Specifically, it is prepared to carry out experiments of the following types:

- Research with rodents: As on Earth, the biological study in rodents allows extrapolating valuable results to the case of the human being. One of the main objectives of this type of research is the study of the molecular mechanisms that involve the bone loss that occurs during exposure to microgravity for a possible pharmacological intervention.
- Acceleration environment: The systems of an orbiting satellite are not all subject to zero gravity, this only happens in the centre of mass of the satellite, which does not always correspond to a physical point of the same. In the ISS, it is possible to carry out studies of disturbances due to this phenomenon, which are transmitted mechanically as vibrations through the structure of the vehicle, or acoustically through the air inside the habitable modules.
- Macromolecular crystalline growth: Most of these cultured crystals are proteins, although there are also DNA and even whole viruses. In this way, it is possible to study more in depth their operation and the interaction between them.
- Microbial research: The number of microbial inhabitants in a human being exceeds in 10 the number of cells of the same, most of them beneficial to their host or simply innocuous. Given the interest in space exploration, it is extremely important to study the effects of the space environment of these microorganisms in humans, since either the change of environment or the possibility of invasion of them in other locations within the organism can cause become pathogenic.
- Human research: The ISS provides an exclusive opportunity to advance in the investigation of the effects of the environment during a long-term space flight in humans, so that physical, pharmacological and nutritional resources can be developed that guarantee the health of the crew.
- Technological demonstration: The ISS provides an infrastructure capable of demonstrating operational concepts, prototypes and systems that could be included in future space flights, and can be evaluated without significant danger to the crew and the vehicle, reducing risks of future exploration missions.
- Fluid Physics: Study of the movement of liquids and gases, whose environment makes it possible to reach conclusions that could not be reached in any other way, and whose results are applicable both on Earth and in a space environment. Motivated fundamentally by the fact that almost all the vital, environmental and biological support takes place in the fluid phase.
- Combustion science: The objective of studies of combustion in microgravity is the best predictive quantitative understanding of this process, since they can be reduced to a much simpler one-dimensional process.
- Earth Observation: Some objectives of these studies are to improve the understanding and predictability of phenomena such as the ozone layer, meteorology, climate change, carbon cycles or the terrestrial magnetic and gravitational field.
- Cell Biology: Has two fields of research, on the one hand, the compression of the fundamental mechanisms of response to changes in gravity, and on the other hand, the use of it as a tool to make advances in tissue applications.
- Fundamental Physics: The ISS also allows experiments in the field of physics, such as prolonged exposure to free fall, the simplest measurement of potential changes in gravity and relative movements, the study of very small accelerations in celestial bodies or the study of propagation of optical and radio signals as the atmospheric interference is reduced.
- Effects of the space environment: Study of the effects of the space environment on the materials used on the outside of spacecrafts. The threats to which they are exposed are, among others, vacuum, ultraviolet radiation and charged particles, plasma, extreme temperatures and thermal cycles and impacts with micrometeoroids and orbital debris.
- Research of materials in microgravity: The ISS allows the study of microgravity and its effect on materials, associated with heat transmission and mass transport. The station allows for long-term exposures of these materials in a space environment.

For the completion of all these types of experiments, the station has a series of modules, each of them with different characteristics and intended for different purposes. Figure 5 shows an outline of the configuration of the ISS separates into its various modules (in 2007).

Figure 5. ISS configuration in 2007

5.1 ISS orbit

To carry out an experiment in the ISS, and more specifically on the outside, it is necessary to know its orbit around the Earth, so that its conditions can be checked at all times.

A simulation of the orbit has been carried out with the computer software STK (Systems Tool Kit), which allows to verify the regions in which the station receives direct radiation from the Sun and those in which it is eclipsed by the Earth.

To perform the simulation, the orbital elements of the station are needed, which are listed in Table 6.

Table 6. Orbital elements of the ISS

It will be hypothesised that these orbital elements will remain constant, since, due to the presence of the Earth's atmosphere, ISS orbit varies with time. Given that the period to be simulated is very short (a full orbit to the Earth, which is approximately one hour and a half), this effect will not affect the results of the simulation, but it will also be considered that the behaviour of it will be similar to medium-long term, at the time the experiment will be carried out.

In this way, the results of the simulated orbit will now be displayed. The period that has been simulated is approximately from 10:00 to 11:30 September 1, 2018. This includes, as already mentioned, a complete orbit around the Earth, where the time in which the station remains eclipsed by the Earth and the time in which it receives direct radiation from the Sun will be verified.

First, Figure 6 shows the initial simulation time, at approximately 10:00. At this moment, the station is in a brief period of semi-darkness, when it "sees dawn", and the period in which the station begins to receive direct radiation from the Sun.

Figure 6. ISS orbit. 10:00 09/01/2018

In the next point, represented in Figure 7, an intermediate point of the section in which the station receives direct solar radiation is observed. The position of the Sun can be inferred from the position of the solar panels (the model is configured so that they follow the Sun). This point corresponds to 10:30, and it is observed as the whole station receives the solar radiation through its orbit.

Figure 7. ISS orbit. 10:30 09/01/2018

At approximately 11:00 the station enters the eclipse zone, again passing through a brief period of semidarkness, corresponding to the "sunset" at the station. This fact can be checked in Figure 8.

Finally, in Figure 9, you can see how the end of the eclipse zone is reached and, thus, the end of the orbit that has been analysed. This occurs at nearly 11:30.

Approximately, the orbit of the station lasts about 90 minutes, of which for about 60 minutes it receives direct radiation from the Sun. It daily performs about 15 and a half orbits (since the orbital period is somewhat longer than 90 minutes, around 93 minutes), therefore, the ISS is subject to about 15.5 hours of solar radiation per day.

It is verified through the simulation that there is no point of the ISS that remains permanently protected from solar radiation, so it will be necessary, as will be developed later, the use of a method of protection against it.

Figure 8. ISS orbit. 11:00 09/01/2018

Figure 9. ISS orbit. 11:30 09/01/2018

In this chapter the Japanese Module of the ISS will be described. This has been suggested by Chris Barber, founder of ISSET, to the author of this project as a possible optimal place for the fulfilment of the experiment.

6.1 Kibo

The Japanese Experimental Module (JEM), known as Kibo, is the contribution of the JAXA (Japanese Space Agency) to the International Space Station. It is a pressurised module, designed to carry out scientific research activities in orbit, with a capacity of up to four astronauts. At the moment, a great variety of scientific, medical and educational experiments are carried out.

Figure 10. Kibo module integrated into the International Space Station

This module consists of six main elements:

 Pressurised Module (PM): It is the main installation of Kibo. All rack systems required for in-orbit operations are installed in this module. It is, in addition, the largest pressurised module of the ISS.

 Exposed Facility (EF): It provides a multipurpose platform where experiments can be implemented and operate in an environment exposed to space. The payloads can be exchanged or retrieved using the Kibo robotic arm.

- Experiment Logistic Module (ELM): It is divided into:
	- o ELM Pressurised Section (ELM-PS): It is an orbiting storage facility that provides room for experiments, samples and spare parts. You have freedom of access and movement between this module and the PM.
	- o ELM Exposed Section (ELM-ES): It is located at the end of the EF, to provide payload space. In addition, it provides logistic functions, since it can be separated from the EF and come back to Earth together with the returning ship.
- Japanese Experiment Module Remote Manipulator System (JEMRMS): Remote manipulator system to support experiments that will be carried out in the EF, or to support maintenance tasks.
- Inter orbit Communication System (ICS): Provides a communication network between Kibo and the Tsukuba Space Centre (TKSC, JAXA).

Figure 11 shows a representation of the module with all the elements just described, and in Figure 12 the overall configuration of the complete installation, with the dimensions of each platform.

Figure 11. Elements of Kibo module

CBM: Common Berthing Mechanism

Figure 12. Diagram of Kibo configuration

6.2 Exposed Facility (EF)

The specifications of this platform provided by JAXA are those shown in Table 7.

Table 7. EF specifications

As described, this platform has some attachment points, in which the payload of the different experiments carried out in it is located. In Figure 13 the location of the different points in a real image can be checked, and in Figure 14 a diagram with the characteristics of each of them.

Figure 13. EF attachment points

Figure 14. EF attachment points diagram

6.3 Environmental conditions

Then, the impacts that the environment has on the payload hosted on this platform will be listed. It is summarised in Table 8.

6.3.1 Thermal conditions

The main objective of this project is to carry out a preliminary analysis of the feasibility of conducting the experiment, but not to carry out a complete design of it (given, in addition, the limitations regarding access to information of the ISS). That is why the scope of the same does not include the performance of a comprehensive calculation of the thermal conditions of the payload.

However, all the possible information is going to be provided in order to carry out the detailed analysis in more advanced phases of the design.

Within the different conditions and disturbances that the payload may suffer during the experiment, thermal conditions are of special interest, given the nature of the experiment that is intended to be carried out. This will not have critical electronic devices, so the effects on them are second order in a first design thereof.

The payload in the EF is exposed to:

- Direct solar radiation.
- Solar radiation reflected by the Earth's atmosphere (albedo).
- Infrared radiation from the Earth (OLR).
- Cosmic microwaves.

For each of these effects, the range of conditions in the area where the payload is housed has been estimated, and can be checked in Table 9.

Table 9. Value of thermal conditions of the payload in the EF

As seen in the previous values, the main effect for the increase in temperature is direct solar radiation, which, as has been verified, is unavoidable throughout the ISS. It is for this reason that it will be necessary to provide the experiment with the necessary protection against solar radiation. In addition to these effects, the impact of the rest of the structures of the ISS must be taken into account.

All these effects, which affect the station intermittently, cause that the range of temperatures reached is between −150℃ and 120℃.

The orbit inclination of the ISS is 51.6°, and sun angle β varies from -75° to 75°, depending on the season of the year (position of the Earth with respect to the Sun) and the precession of the axis of the Earth. Figure 15 shows the definition of this angle and, in Figure 16, an example of a measure of the angle in which the variations of the angle with respect to time are observed, in which the effects can be differentiated by a part, one of greater frequency due to the effect of the precession and, on the other, one of smaller frequency resulting from the position of the Earth with respect to the Sun.

To verify that the simulation carried out previously of the orbit of the ISS on the computer software STK, and also verify that this described trend is followed, the variation of this angle in it has been calculated. In Figure 17 the variation of the angle during a full year can be verified. It is observed that the tendency is the same as that described above.

Figure 15. Solar angle definition

Figure 17. Solar angle variation from 09/01/2018 to 09/01/2019

With these data, together with others from the structure of the ISS itself, it would be possible to perform a detailed analysis of the thermal conditions of the payload during the experiment.

The EF also has a cooling system in almost all the attachment points, in which a coolant is used to have up to a maximum of 6 kW of heat rejection. The objective of the experiment is to demonstrate that no active cooling system is necessary to maintain cryopreserved organic material, however, this system could be useful to mitigate calorific effects from the station itself and thus reproduce as reliably as possible the conditions in space.

6.4 Conducted experiments

Next, some of the experiments that have been carried out on the platform are going to be listed.

6.4.1 *Monitor of All-sky X-ray Image* **(MAXI)**

Composed of two X-ray cameras to control the intensity of them in more than a thousand astronomical sources. Every 15-17 hours the system is monitored by JAXA land centre. It was launched in 2009 and was hosted for two years in the attachment point previously numbered (in Figures 13 and 14) as #1.

Figure 18. MAXI housed in EF (left), and images taken by it (right)

Figure 19. MAXI payload configuration

6.4.2 *Superconducting Submilimetre-Wave Limb-Emission Sounder* **(SMILES)**

The objective of this mission was the observation of submillimetre waves emitted by gases, especially sensitive to stratospheric ozone. Its objective was to eliminate the mechanism of depletion of the ozone layer. It was launched in 2009, but failed at seven months. He was lodged in attachment point # 3.

Figure 20. SMILES in transfer vehicle

Figure 21. SMILES payload configuration

6.4.3 *Space Environment Data Acquisition equipment – Attached Payload* **(SEDA-AP)**

Its purpose was to measure the space environment (neutrons, plasma, heavy ions, light particles of high energy, atomic oxygen and cosmic dust) in the ISS orbit, to investigate the interaction of this with materials and electronic devices in the EF. It was launched in 2009, and housed in attachment point #9.

Figure 22. SEDA-AP payload configuration

6.4.4 *Hyperspectral Imager for the Coastal Ocean* **(HICO)** *& Remote Atmospheric & Ionospheric Detection System* **(RAIDS)** *Experimental Payload* **(HREP)**

Conducted by NASA, HICO takes hyper-spectral maritime images to characterise the coastal regions of the Earth. On the other hand, RAIDS is a remote sensing instrument for ultraviolet and visible rays that measures the electrical density to improve the resistance models in the ionosphere. It was launched in 2009 and lodged in attachment point $# 6$.

6.4.5 *Multi-mission Consolidated Equipment* **(MCE)**

This mission incorporates five instruments, and includes atmospheric observation research that studies the dispersion of rays and plasma across the edge of the atmosphere (IMAP). It also carries out technological demonstration research that consists of the deployment of inflatable structures (SIMPLE), movement of robotic elements (REXJ), and the testing of a high definition television camera (HDTV) in the space environment. Housed in attachment point #8.

Figure 23. MCE payload configuration

6.4.6 *Astrobiology Exposure and Micrometeoroid Capture Experiments* **(Tanpopo)**

It is the first astrobiology experiment carried out in the ISS, and its aim is to evaluate the key points of the hypothesis of "Panspermia". A small platform with a low density solid material called aerogel is exposed and captures solid microparticles, such as micrometeorites carrying organic particles and possible terrestrial particles in low Earth orbit.

At the same time, these organic compounds are kept exposed in space to evaluate their survival and possible alterations, and they are studied in laboratories when returning to Earth.

6.4.7 *CALorimetric Electron Telescope* **(CALET)**

Astrophysical mission that looks for traces of dark matter and provides direct measurements of the highest energy of the cosmic ray electron spectrum to observe discrete sources of high-energy particle acceleration. It lodges in attachment point #9.

Figure 24. CALET payload configuration

7 PROCEDURE OF THE EXPERIMENT

This section describes how the experiment would be conducted, from what cryopreserved material is received to the ISS until it returns to Earth and is ready to reheat it.

In this procedure, those aspects that directly affect the space mission will be collected and not the way to cryoconserve and re-heat the samples, which are supposed to be well-known procedures.

In this way, the following phases are contemplated for the fulfilment of the experiment, which will be described below:

- 1. Payload.
- 2. Safety.
- 3. Launch.
- 4. Installation.
- 5. Return.

7.1 Payload

The first phase consists of, according to the payload that it is necessary to send to the station, to carry out its design based on the capabilities of the platform where it will be hosted, and which have already been described above.

7.1.1 Standard structure of the payload of the EF

The Kibo EF has a predefined structure in which the payload must be housed in order to perform the experiments. This structure allows the different attaching and de-attaching operations on the platform, operated, as will be detailed later, by the robotic arm of the module (JEMRMS).

Figure 25. Container structure of the standard payload for the EF

The characteristics of this box are the following:

- Size: 0.8 x 1.0 x 1.85 m
- Mass (Weight): Up to 500 kg

Figure 25 shows a representation of this structure, with all the components necessary to operate on the platform and not related to the experiment itself.

JAXA allows higher loads, with a different box designed for this purpose, however, it is restricted to only some anchor points and under an in-depth study of the interference of the structure with the operative envelope of the robotic arm, the equipment and the Chambers of the platform, the EVA's (exits of the crew to the outside) that can be conducted and the rest of the experiments.

In this case, the size of the experiment will be small enough to be able to be housed in the standard structure, so there are no compatibility problems with the platform.

Figure 25 shows the different parts that make up this structure, which are described below:

 HTV Cargo Attachment Mechanism – Passive (HCAM-P): It allows attaching the structure to the transfer vehicle (HTV), through the HCAM to the support element of these containers, called Exposed Pallet (EP). The details of this system can be seen in Figure 26, and the actual coupling between HCAM-P and HCAM in Figure 27.

Figure 26. Detail of HCAM-P and HCAM system

Figure 27. Coupling between HCAM-P and HCAM

 HTV Connector Separation Mechanism – Passive (HCSM-P): It is the mechanism, together with the corresponding one housed in the HTV (HCSM), that allows the separation of the payload container from the vehicle. A detail of the system and its operation can be verified in Figure 28.

Figure 28. Detail of HCSM-P and HCSM system before (left) and after (right) separation

 Payload Interface Unit (PIU): Mechanism for coupling in the EF, both structurally and to receive resources such as electrical and thermal power and communications. The equivalent mechanism in the EF is called the Exposed Facility Unit (EFU) and together they are called the Equipment Exchange Unit (EEU). In Figure 29 a detail of both mechanisms can be observed.

Figure 29. EEU mechanism

 Grapple Fixture (GF): Gripping mechanism of the robotic arm from the platform to the structure. In Figure 30 the configuration of it can be seen in real containers.

Figure 30. Grapple Fixture

7.1.2 Payload configuration

In this section, first, the payload that will be part of the experiment will be determined and then it will be housed in the structure defined above.

As already indicated, the objective is to transport the EEI and to house in it a set of samples of biological material, such as germplasm, of animal or vegetable species. The way to transport them will be by means of straws that are normally used for transport and storage in a cryoconservation centre on Earth.

7.1.2.1 Transportation at cryogenic temperatures

Samples must remain at cryogenic temperatures at all times, from before launch until after return, so, at least until they are coupled to the space station, they must remain refrigerated by some means.

Next, the different feasible options for the maintenance of the samples at cryogenic temperatures during the transport to the ISS will be developed.

7.1.2.1.1 Dry transport containers

One of the possible options for transporting the samples to the ISS at cryogenic temperatures is the use of dry transport containers, known as Dry Shippers. These tanks have a porous material inside that absorbs liquid nitrogen and allows the vials to maintain the biological material at cryogenic temperatures.

In the market, there are several types of containers, among them, you can differentiate two main types, one of them reusable, made of metal and, in general, of greater capacity and autonomy, and another that are singleuse, made of lighter materials, smaller sizes and autonomy.

The latest advances in single-use canisters have allowed them to become reusable, changing, among other things, the material that absorbs liquid nitrogen.

Table 10 shows the main characteristics of the two types of it.

Table 10. Dry Shippers characteristics

The types of canisters shown also comply with the aeronautical regulations of IATA.

7.1.2.1.2 Cryogenic freezers

In addition to the aforementioned dry transport canisters, portable freezers also stand out. These devices allow samples of biological material to remain stored at cryogenic temperatures for a longer period.

These freezers are characterised by maintaining the samples at temperatures of -80 ° C without the need to use refrigerants, such as liquid nitrogen or compressors. However, they require the use of power supplies.

The mechanism of operation of these devices is based on the Stirling cycle, a thermal engine whose base of operation is the compression and cyclic expansion of a gas at different temperature levels to achieve a conversion of thermal energy into mechanical energy. These engines are characterised because they have a high efficiency and great ease to be applied to any source of heat. Nowadays, these advantages offer an interest in the use of this type of devices.

Table 11 shows standard models of this type of device.

Table 11. Cryogenic freezers characteristics

7.1.2.1.3 Choice of storage type

As will be described later, the duration of the flight to the ISS is around 3-4 days, so the time it will be necessary to use these devices is short. That is why the best option is the use of dry transport canisters, which would offer the following advantages with respect to cryogenic freezers:

- It is a passive sample conservation system, that is, it does not have to be supplied with electrical energy for its operation, which makes the cost and complexity of the installation lower.
- Price is much lower than in the case of freezers, which implies a significant reduction in the total cost of the mission.

Of the two options that have been commented, the use of Dry Shipper 3.0 would imply a series of advantages in relation to Artic Express Dry Shipper:

- Being made of fibres instead of metal is lighter, decreasing the weight of the canister.
- The cost is much lower than that of its alternative, at the cost of a lower autonomy, but that is sufficient for the time that the flight will last.
- With the new versions the disadvantage of not being reusable is removed.

This is why Dry Shipper 3.0 system will be used to store and maintain samples at cryogenic temperatures until they are coupled to the ISS. These canisters include a storage system to house the straws with the biological material.

Once the container is coupled in the ISS, and after exhausting the autonomy of canisters, straws will remain for the duration of the mission housed in them, which will no longer be responsible for maintaining the cryogenic temperatures, but it will be the space environment.

7.1.2.2 Accomodation in the container

Once that the type of storage in which the samples are to be transported is selected, the next step is to define the storage of these in the standard container for payloads in the EF, described above.

To do this, a CAD model has been made, so it has been decided to optimise the number of units that will be transported so that they are as much as possible. Figure 31 shows a representation of the organization of the same within the container, while Figure 32 shows a dimensioned drawing.

Figure 31. Representation of the distribution of canisters inside the container

Figure 32. Plane of the distribution of canisters inside the container (dimensions in mm)

It is verified, therefore, that the maximum number of canisters that the container would allow lodging is twenty. This implies that about 4000 straws of 0.25 mL, or 2000 of 0.5 mL (or a combination of both) could be used in the experiment.

The proposed way to fix canisters to the container is with foam moulds, on two floors, to minimise vibrations and impacts during the flight. There would also be room or, otherwise, a canister could be replaced with some additional system, such as temperature sensors to control it during the development of the mission. As Kibo supplies electrical power to the container, it would not be necessary to include any power generator in it.

With this payload, the weight of it would be approximately 102 kg, plus the weight of the foam, so that there is a sufficient margin with respect to the maximum, 500 kg.

7.1.2.3 Radiation shield

One of the most important aspects is the protection of the payload against solar radiation, and other sources of heat that can increase the temperature of the samples above the admissible ones.

It has been previously verified that from the position in which the container attached in the ISS is to be found, it is impossible to avoid direct radiation from the sun, so the only solution is to provide it with a shield that reflects all of it and manages to maintain inside the desired temperatures.

The purpose of this project is not to carry out the exhaustive design of all the systems that would be part of the mission, therefore, in the case of the shield, a solution whose viability has already been studied to integrate it into a mission that requires similar characteristics is going to be proposed.

This mission is the James Webb Space Telescope, which is discussed in more detail in the other part of the project. The only noteworthy feature in this case is that the systems of the telescope must be at very low temperatures, around −223℃, so that the shield that is going to be used in said mission must be able to reflect practically all radiation it receives.

This shield is made up of five thin layers, to prevent the transmission of heat by conduction. Between them, the existing vacuum acts as an insulator, favouring to a greater extent the temperature drop between each of the layers. Figure 33 shows the scheme and the operation of the shield.

Cross-Section of Webb's Five-Layer Sunshield

Figure 33. Shield planned for use in the James Webb Space Telescope

Studies that have been made conclude that, once the telescope in orbit, while the first layer of the shield would be at 109.85℃, the inner layer would be at a temperature of −237.15℃.

The different layers that form the shield are constituted by kapton, a material that stands out for its lightness and its thermal properties. It is characterised by high heat resistance and remains stable in a fairly wide range of temperatures ranging from -269 °C to 400 °C.

As regards surface treatments, all layers are treated with aluminium and the first two, which are more exposed to solar radiation, are also treated with doped silicon. These materials have good properties in the space environment, do not degrade easily and favour the emission of light and heat.

The idea is, therefore, to cover the payload container of this protective shield, so as to minimise the effect of radiation, both solar and other bodies that may affect it.

7.2 Safety

Every engineering project must be subject to rigorous safety studies to ensure the integrity of the systems and users. In the space field this fact is especially critical, for two fundamental reasons in the case of the ISS.

In the first place, due to the high cost of the projects, a failure in a rocket launching means millions in economic losses, in addition, failures that imply problems in the station or its modules are even more important. On the other hand, a failure in the ISS implies endangering crew members, so the safety measures must be especially severe and be subject to numerous controls throughout the design process.

As it has been defined, the scope of this project does not include the complete design of the mission, with all the factors that this implies, so it is not possible to carry out a detailed safety study that includes all the elements of the experiment. However, here are going to list the different documents, prepared by the different entities responsible for carrying out missions to the station. In this way, the completion of the complete safety study in more advanced phases of the design is canalised, when the characteristics and systems that are going to be part of it are well delimited.

In missions to the ISS, there are two types of safety studies that must be carried out:

- Payload safety study: The developer of the payload must ensure that any danger to the ISS, Kibo module or crew members is avoided. It must be taken into account, as has already been stated, that danger does not only affect the success or failure of the experiment, but also any failure that affects the rest of the systems and personnel of the station.
- Mission safety study: Depending on the type of mission that is to be carried out, a document must be drawn up certifying that monitoring and verification has been carried out during all the phases that comprise the design of the mission.

7.2.1 Payload safety

Safety documents are associated to each phase of the mission, which are listed below:

- HTV:
	- o *JMP-002B: Rocket and Payload Safety Standard*
- Progress and Soyuz (Russian vehicles):
	- o *P32928-103: Requirements for International Partner Cargoes Transported on Russian Progress and Soyuz Vehicles*
- ISS (non-Russian modules):
	- o *SSP-51700: Payload Using the International Space Station*
- ISS (Russian modules):
	- o *P32958-106: Technical Requirements for Hardware to be Stored or Operated on the ISS Russian Segment*

As it is verified, safety regulations to follow differ according to whether or not Russian vehicles and facilities will be used. As will be explained below, in the development of the mission that is being considered, these documents will not be used, so the documents referring to non-Russian vehicles and modules will be applicable.

With regard to the payload, there are some elements considered potentially dangerous that must be taken into consideration during the design and development of the same. Next, they will be listed, indicating also the additional regulations to the previous ones on which each of them is supported.

- Structural damage: The payload must be designed to avoid damage to the ISS or Kibo module, in case of structural damage. The use of materials and parts must be controlled, defining a safety factor for the load during launch, emergency landing, EVA, etc.
	- o *SSP52005 ISS Payload Flight Equipment Requirements and Guidelines for Safety Critical Structures*
- Moving parts: Any equipment that includes rotating parts, such as a motor or axle, must be designed to avoid unexpected direct contact with the crew, the ISS or Kibo.
- Contact temperature: Temperature should be regulated to avoid damage to spacesuits and crew due to unexpected contacts in EVA activities.
	- o *NASDA-ESPC-840 JEM System Specification*
	- o *NSTS 07700 Volume XIX Appendix 7*
- Sharp edges: The safety of the crew during the EVA must be guaranteed for any catastrophic damage caused by sharp edges. In this way, the corners or edges must be chamfered.
	- o *SSP50005 ISS Flight Crew Integration Standard*
	- o *NSTS 07700 Volume XIX Appendix 7*
- Pinching: ensure that the crew does not suffer snags or pinching with flexible areas during the EVA.
- External contamination: The payload must be designed not to emit any hazardous material in the launch vehicle or in the ISS. In addition, the average annual amount of exhaust gases from the material of the payload must be evaluated and estimated.
- Flammability: Although the payload in the EF is installed in outer space, regulated materials must be used to prevent fires at the launch site.
	- o *NHB 8060.1C Flammability, Odor, Offgassing, and Compatibility Requirements and Test Procedures for Materials in Environments that Support Combustion*
	- o *NSTS 22648 Flammability Configuration Analysis for Spacecraft Systems*
- Glass pieces: Any glass part must be covered to avoid damage to the crew during the EVA in case it breaks.
- Pressurised and sealed containers: Their safety must be tested by fatigue analysis test according to the pressure regulated in *JDX-2012303 NC*.
	- o *ANSI/AIAA S-080-1998 Space Systems – Metallic Pressure Vessels, Pressurized Structures, and Pressure Components*
	- o *ANSI/AIAA S-081-2000 Space Systems – Composite Overwrapped Pressure Vessels (COPVs)*
- Electric Shock: The payload must be designed with a ground connection and to avoid electric shock to the crew by contact with high voltage equipment.
	- o *SSP30240 Space Station Grounding Requirements*
	- o *SSP30245 Space Station Electrical Bonding Requirements*
- Electrical circuits: They must be designed to have protection circuits, such as a current limiter or a fuse, to prevent failures in the event of a short-circuit. It must also be ensured that two or more hazard control functions are prevented from being invalidated by a short-circuit.
- Electromagnetic Compatibility (EMC): Payload must be designed to avoid any malfunction of itself or other equipment caused by electromagnetic interference.
	- o *SSP30237 Space Station Electromagnetic Emission and Susceptibility Requirements for Electromagnetic Compatibility*
- Optical instruments: Optical instruments that have harmful light intensities and wavelengths should be designed to prevent them from affecting the crew unexpectedly.
	- o *ANSI-Z-136.1 American National Standard for Safe Use of Lasers*
- Gas leakage: The exhaust gases from the PM and EF payloads must be absorbed during the EVA to prevent the crew from becoming contaminated.
-
- Batteries: The battery cells for the payload must be designed and verified as non-flammable and to prevent corrosion and the release of toxic gases. In addition, it must be guaranteed that damage due to faults such as abnormal temperatures, short-circuits, reverse current, reverse connection, leakage and excess voltage is avoided.

7.2.2 Mission safety

This section details the process of verification of the mission consisting of conducting an experiment in the Kibo module, since this is the case in this project.

In this case, first, the person in charge of carrying out the corresponding verifications is the developer of the payload. Next, JAXA receives the results of these verifications and prepares a report based on them, after which NASA receives it for final verification.

As a result of this process, a document called Safety Assurance Report (SAR) emerges, which is updated with each step in the design process. This document is based on the following reference documents:

- *JSX-2008041A: HTV cargo review process*
- *NSTS/ISS 13830C: Payload Safety Review and Data Submittal Requirements For Payloads Using the International Space Station*
- *SSP-30599E: Safety Review Process (for the Russian Vehicle and module)*

Apart from the safety study of the payload, included in the global safety study of the mission, the rest of the elements and procedures are going to be those established standardly by these entities, so it can be assumed that all the requirements of necessary safety are going to be fulfilled.

However, to illustrate the process of verification of the design of a mission in the ISS, the different phases of this process are listed, with the purposes pursued by each of them.

- Phase 0:
	- o Identify dangers.
	- o Establish safety requirements.
	- o Evaluate safety.
	- o Verify that all the above has been incorporated into the preliminary design.
- Phase 1:
	- o Confirm that all elements of the preliminary design meet the safety requirements.
	- o Verify that hazard control and methods for verification are adequate.
	- o Incorporate all the results of this phase into the design.
- Phase 2:
	- o Confirm that the entire design meets design requirements.
	- o Verify the implementation of risk controls and their verification methods in the design.
	- o Verify that the above has been incorporated into the design.
- Phase 3:
	- o Confirm that the safety verification and all the objectives listed above have been completed.
	- o Confirm that all actions to be performed on elements are closed.
	- o Approve the final version of the SAR and evaluate the final safety.

7.3 Launch

7.3.1 Pre-launch operations

After development phase and completion of all control and verification tests, payload is stored in a container to be housed in the transfer vehicle.

To perform the final inspection before launch, containers are sent to the Tanegashima Space Centre (TNSC), where the payload is unloaded and moved to a clean room. There it is given visual inspections and telematic and command controls.

After this, it is sent to the centre where the launch is made, depending on the vehicle used, where checks will also be made just before installing them in the vehicles and proceed to the launch.

7.3.2 Launch

After the removal of the Space Shuttle in 2011, vehicles used for transport to or from the ISS are those listed in Table 12.

Vehicle	HTV (JAXA)	ATV (ESA)	Progress (Russia)	Cygnus (NASA)	Dragon (NASA)	
First launch	2009	2008	1978	2013	2010	
Number of flights	$\overline{7}$	5	161	10	17	
Capacity	7t	7.5t	2.5t	2.7t	Launch	6 ^t
					Return	3 _t
Mass (Weight)	16.5t	20.5t	7.3t	5.3t	10.2 t	

Table 12. Transfer vehicles to the ISS

7.3.2.1 ATV

ATV (Automated Transfer Vehicle) is the unmanned vehicle of the ESA, which assumes the functions of supply, waste disposal and periodic lifting of the ISS. It is planned to make a modification to allow the re-entry for material and crew of the ISS, but at this moment it does not have that capacity.

In addition, this vehicle docks in the Russian module, therefore, it is not possible to transport and install the non-pressurised payload in Kibo EF.

7.3.2.2 Progress

Progress is the unmanned vehicle of the Russian Space Agency. It is responsible for bringing food and other equipment for the crew, oxygen and fuel. This vehicle does not have the capacity to re-enter, as it burns in the atmosphere with the rubbish that comes from the ISS.

In the same way as the ATV, this vehicle is coupled in the Russian module and, therefore, it is not possible to use it for the transport of unpressurised payload to install it in Kibo EF.

7.3.2.3 Cygnus

Cygnus is one of NASA's unmanned vehicles, designed to transport supplies to the ISS after the end of the Space Shuttle. This vehicle is not designed for the transportation of the EPs that include the containers with the non-pressurised payload. A new version of the ship is being designed to transport non-pressurised cargo, but at the moment it is not possible.

This vehicle also does not have the capacity of re-entry on Earth, but it is destroyed in the atmosphere with waste from the ISS, as is the case with the Progress.

7.3.2.4 Dragon

Dragon is a reusable vehicle, developed by SpaceX and operated by NASA, with the capacity to fit into the non-Russian segments of the ISS and performs refuelling operations and crew transport that previously made the Space Shuttle fleet.

This vehicle allows the re-entry of payload maintaining the integrity of the same, within a period of about two days from the departure of the ISS. On the other hand, it does not contemplate for the moment the accommodation of non-pressurised cargo of the size of the containers operated by EF, therefore, it is not valid for the transport of the load of payload to the ISS in this case.

The option of returning the experiment payload to Earth is contemplated through this vehicle, since it is not very bulky or heavy.

7.3.2.5 HTV

HTV (H-II Transfer Vehicle) is the unmanned transfer vehicle of JAXA. It is not capable of returning the load to Earth, but it is possible to simultaneously transport both pressurised and non-pressurised cargo to the Pressurised Module and the Exposed Installation of Kibo, respectively.

In the latest version of this vehicle, the HTV-7, also known as Koutonori 7, has included a small re-entry capsule called HTV Small Re-entry Capsule (HSRC). The launch of this vehicle was made on September 22, 2018 and it is expected to be a first step to a major re-entry module, the HTV-R.

After analysing the rest of vehicles, it is concluded that this is the only one in which, based on the configuration developed to carry out the experiment, the launch of the payload can be carried out up to the ISS. Therefore, the characteristics of this vehicle will be developed in more detail.

7.3.2.5.1 Launch vehicle

HTV is launched from TNSC, on board the H-IIB, specifically designed to launch the HTV. In Figure 34 the configuration of this rocket can be verified, with the HTV already inside and the different phases of the fuel for propulsion.

The opportunity to launch this rocket is once a day, since it must be scheduled for when the orbital plane of the ISS passes over the TNSC.

This rocket injects the HTV into an elliptical orbit with an altitude of 200 km of perigee and 300 km of apogee, and an inclination of 51.6°.

Once the separation of the HTV with the H-IIB happens, moment that is reflected in Figure 35, the following takes place:

- HTV subsystems are automatically activated.
- The vehicle maintains its attitude.
- A self-check of the vehicle components is carried out.
- Communications are initiated with the HTV Control Centre at Tsukuba Space Centre (TKSC).

Figure 34. H-IIB general configuration

Figure 35. HTV and H-IIB separation

7.3.2.5.2 HTV flight profile

After the separation of the H-IIB, HTV begins the phase of the approach to the ISS, which lasts about three days, and in which the vehicle is adjusting its orbit, until reaching the Approach Initiation point (AI Point) and starts the proximity operations, which will be detailed later, until being attached to the ISS, where mission operations will begin.

After concluding all of them, the HTV, already loaded only with remains of litter from the station, starts the separation operations of it, until it is destroyed during the re-entry into the atmosphere.

In Figure 36 all phases of the HTV flight profile appear.

Figure 36. HTV flight profile

7.3.2.5.3 Configuration

The general outline of the vehicle can be seen in Figure 37, in which its main components are indicated:

- Avionics Module (AM): Includes navigation, communications and electrical equipment.
- Propulsion Module (PropM): Includes necessary fuel to perform all operations during the mission, such as, for example, hooking up to the station, attitude control, etc.
- Common Berthing Mechanism (CBM): Used to attach the vehicle to the ISS.
- Pressurised Logistic Carrier (PLC): This module houses the pressurised charge.
- Unpressurised Logistic Carrier (ULC): This module houses the non-pressurised payload, included in the containers described above attached to the EP.

Figure 37. HTV scheme

Thanks to this modular design it is possible to have different vehicle configurations, depending on the specific needs of each mission. Thus, in Figure 38 some possible configurations are shown, and in Table 13, capacity in each one of them.

Number	Type	Load to the ISS	
$\bf{0}$	Original vehicle (Figure 37)	4.5 t of pressurised load 1.5 t of unpressurised load	
1	Only pressurised load	7.0 t of pressurised load	
$\overline{2}$	Only unpressurised load	7.0 t of unpressurised load	
3	Pressurised load and re-entry capsule	4.5 t of pressurised load 2.5 t of capsule system	

Table 13. HTV loading possible configurations

Figure 38. HTV possible configurations

Configuration (3) shown would correspond to a version of the vehicle with a re-entry capsule, as it is currently being developed.

Exposed Pallets (EP), where containers that include the unpressurised payload are attached, can also be adapted in different configurations, as shown in Figure 39. With the configuration shown on the left in the figure it is possible to transport up to three containers with payload. With the configuration on the right, both external equipment and batteries can be loaded.

Figure 39. EP configurations

The figure shows some previously described parts of the containers, as in the case of the HCAM hooks, the GF, which is where they are attached to the robotic arm of Kibo or PIU, which is the attachment to the structure of the EF. In EP there is another port, Power Video Grapple Fixture (PVGF), which is the place where one of the robotic arms of the ISS, not of Kibo in this case, will be attached to them during payload installation in the EF.

7.4 Installation

This phase begins when the HTV begins its phase of proximity operations at the Approach Initiation point (AI), 7 km from the ISS. The operations carried out by HTV during this phase, which can also be checked in Figure 40, are the following:

- HTV moves from AI to a point 500 metres from the ISS, guided by relative navigation based on GPS.
- Using a laser radar called Rendezvous Sensor (RVS), HTV approaches the station.
- During the approach, HTV stops at two points, one at 300 metres, called a Waiting Point, and another at 30 metres, called Parking Point.
- Finally, HTV approaches the coupling point, 10 metres from the ISS.

Figure 40. HTV proximity operations

Once the HTV arrives at the coupling point, it is connected to the ISS through one of the robotic arms of the station, the Mobile Maintenance System (MSS), known as Canadarm2. In Figure 41 the final phase of the HTV approach can be seen and, in Figure 42, it is already coupled to the Canadarm2.

Figure 41. Final approach of HTV to the ISS

Figure 42. Coupling HTV to the ISS through Canadarm2

Once attached to the mechanical arm, the PLC is coupled to one of the modules of the station, to allow the passage of the crew. On the other hand, Canadarm2 is attached to one of the EPs of the ULC and extracts it from it. This operation is represented in Figure 43.

Figure 43. Removing the EP from the unpressurised HTV module

The following sequence of operations is shown in Figure 44, and consists of:

- 1. After removing the EP from HTV, it moves to the proximity of Kibo EF.
- 2. The EP is attached, through its GF, to the robotic arm of Kibo, and is uncoupled from the Canadarm2. This operation can also be observed in Figure 45.
- 3. Kibo robotic arm moves EP to the proximity of EF.
- 4. EP is attached to berthing point # 10 (as defined in Figures 13 and 14) through PIU and decoupled from the robotic arm.

Figure 44. Attaching process of EP to Kibo EF

Figure 45. Installation process of payload in EF

Once the EP is attached to the EF, the robotic arm can be coupled to one of the containers, through the corresponding GF, and transferred to the berthing point provided during the design of the mission, attached to it through PIU.

7.5 Return

As described above, the return to the Earth of the payload must be made in a vehicle other than the one of arrival, since the HTV does not have the option of re-entry. In this case, it could be done in Dragon of NASA.

For payload recovery, it could be done either through Kibo PM or, if that option is not available, performing an EVA.

The samples, contained in straws housed in canisters, as already described, may be returned to Earth housed in them, since, being reusable, it would be enough to fill them with liquid nitrogen, after which they would count again with full autonomy, 3-4 days, enough time for the return. The nitrogen supply can come either from the ISS tanks themselves or from an additional supply that can be sent during the mission.

Another option would be to lodge straws in cryogenic modules of the return capsules, in case of having them. This will depend on the availability of the same at the time when the return of the samples after the end of the mission is to be made.

7.6 Alternative procedures

In this section a viable procedure with current technology and means has been developed, to corroborate that, indeed, it would be possible to carry out the experiment. However, in recent years there have been advances in the technology of both transport vehicles, as has been shown above, and in the ISS modules themselves.

In Kibo module itself, it is being studied to expand the range of workable experiments. One of the options is the inclusion of experiments in small modules that could be attached to the robotic arm during the whole mission, if its duration is not long, or orbiting next to the station with the possibility of rescuing it through the arm when the mission has ended.

These procedures have not yet been defined, therefore, today it would not be possible to carry them out, but, if the necessary advances were made to be possible, they would entail various advantages, which are presented below:

- Ease of transport: Since it is no longer necessary to incorporate the payload to the containers described above, it is possible to transport it in a small module in virtually all transport vehicles, providing much greater versatility to it.
- PM check: Before proceeding with the coupling of the module in the robotic arm (or releasing it in orbit), in the Pressurised Module a final inspection could be made and the good state of the payload after transporting. In addition, it could be maintained in the pressurised modules of the ISS if it was necessary to carry out examinations or problems arose in any of the systems necessary to carry out the mission.
- Return to Earth: Since payload has a small size and returns to the PM when the experiment ends, the ease of transferring it to a vehicle that performs re-entry is much greater.
- Greater versatility during the experiment: If the experiment is attached to the robotic arm, with the ability to move, it would be possible, for example, to design the mission to minimise the direct impact of solar radiation.

Figure 46. Alternative procedure in Kibo, where a small module remains attached to the robotic arm
After the development of the project, it is possible to conclude that, at least technically, it would be viable to carry out an experiment of these characteristics in the International Space Station. It has been verified that all the procedures can be based on similar ones already carried out and, therefore, and as already mentioned, they are technically safe to carry out.

For other systems that have been proposed external the ISS, the choice has been based on the proven reliability of the same, as in the case of dry transport canisters, or in studies carried out by organizations such as NASA, such as the protection against radiation.

However, this is still a first development of an experiment that must undergo a much more comprehensive design. Next, some developments that do not fall within the scope of this project are going to be considered, but which should be taken into account in future developments:

- It is necessary to fulfil a study of the economic feasibility of the experiment, which has not been able to develop due to the difficult access to this information. For this purpose, the economic study has been presented for the case of the use of liquid nitrogen on Earth, which would have to be compared with the cost of maintaining samples in space a certain time, and thus obtains the minimum time that should be kept samples in orbit to amortize the development, launch and return expenses.
- Procedures should be updated according to the latest capabilities of the ISS. Manuals that have been accessed date approximately from the year 2010. Although, in general, development will be the same, it is possible that modifications have been made to the modules in a way that makes it much more efficient fulfilment of the mission, or may be carried out following another procedure, such as those presented as alternatives in the project.
- It is also essential to carry out a detailed thermal study of the container where the samples are housed. For this, it is necessary to know at all times the amount of radiation that receives both from the Sun and the rest of bodies, as well as the heat that it receives from the station itself. Although not all of these data have been accessed, the most significant concepts have been introduced in order to calculate the amount of radiation received from the Sun, as well as the characteristics that the shield could have.

Although the project has focused on the cryopreservation of genetic material, the possibilities of this field go much further, as explained above.

The authors of this project and the other part of it have verified how both ISSET and cryonics companies, such as KryoRus, with whom the project's tutor has maintained contact, have stated that there is a real interest in carrying out this type of experiments, not with small vials with biological samples, but with more ambitious objectives, such as the shipment of cryopreserved human bodies.

REFERENCES

Luz Mábel Ávila-Portillo, Bacter et al. (2006). Fundamentos de criopreservación. *Revista Colombiana de Obstertricia y Ginecología Vol.57 No.4*, 291-300.

External Payloads Proposer's Guide to the International Space Station, Goddard Space Flight Centre, NASA, 2016

Kibo Handbook, September, JAXA, 2007

Kibo Exposed Facility User Handbook, JAXA, 2010

Development Plan for Future Mission from HTV System, Hiroshi Sasaki, Takane Imada, Shinichi Takata

Study of criogenic storage on the moon, Arthur D. Little

https://www.heraldo.es/noticias/suplementos/tercer-milenio/investigacion/2017/06/21/criopreservacion-unaesperanza-vida-sin-evidencia-cientifica-1182722-2121029.html

<http://criopreservacion.blogspot.com/2007/05/historia-de-la-criopreservacin.html>

<https://www.altima-sfi.com/es/tanatopedia/crionica/>

<http://www.cryonics.org/about-us/>

<https://www.frozenark.org/>

http://www.nhm.ac.uk/discover/news/

http://www.mncn.csic.es/Menu/Investigacion/Serviciodeapoyoalainvestigacin/BancodeGermoplasma/

<http://www.cryodiffusion.com/>

https://www.nasa.gov/mission_pages/station/research/experiments

<http://iss.jaxa.jp/en/kibo/>

<https://www.space.com/18175-moon-temperature.html>

<https://lunar.gsfc.nasa.gov/>

<https://www.diviner.ucla.edu/>

http://www.huvesearch.com

<https://www.laboratory-equipment.com/>

<https://ua.all.biz/en/vertical-deep-freeze-low-temperature-cryo-porter-g490592#.W9Qv23szapo>

<https://www.socalbiomed.com/equipment/freezers-refrigerators/waverly-arctiktm-mini-ult-freezers-90-c.html>

<https://www.fryka.de/en/standard-equipment/cold-boxes/cold-box-b-35-85>

<http://www.arctiko.com/products/86-c-ultra-low-temperature-freezers/dp-80-cryo-porter/>

<http://taurustradinginc.com/cryo-porter-cs-80c/>

<https://www.kisker-biotech.com/frontoffice/product?produitId=0O-109-04>

<https://jwst.nasa.gov/sunshield.html>

GLOSSARY

