



## Agrospace Experiments Suite (AES)

This experiment actually consists of two separate experiments: an education oriented experiment "Space Beans for Students" and a biology experiment "Seedlings".

### 1. Space Beans for Students

The aim of this part of the experiment is to involve and interest children in space science to help with the continuous exploitation of space technology and its application to every day life on Earth. To this end the experiment will consist of beans being germinated in space at the same time as being germinated by students in classrooms on Earth.

The germination of seeds in space has been previously demonstrated but the aim here is to have a good involvement of students in the space mission and increase their knowledge of space, both in terms of what the space environment is and in what the potential applications of the space technology are.

Most students are already aware that plants begin their life cycle when a dormant seed germinates and a seedling begins growing. In this activity, students investigate the conditions that lead to the germination of seeds and observe in parallel the early stages of the plant life cycle on earth and in space.



Space Beans for Students experiment. Plastic bag containing paper towel, seeds and the water container (Image: M. Casucci)

The main purpose of the experiment is to provide the student with answers about the following key questions:

What do plant seeds need to germinate?

What changes do seeds and seedlings undergo during germination and early growth?

What are the main differences between seeds germinating on Earth and those germinating in space in weightlessness?

### How is it done?

The experiment consists of a transparent plastic bag, which is hermetically sealed. Inside the bag is a paper towel on which six bean seeds and a water container are taped.

During 7 days on-orbit the beans will germinate in this bag. The germination will start when the astronaut opens a water container inside the transparent plastic bag. Starting on the first day, a digital photo of the germinating seeds will be taken by the astronaut. This will occur at the same time every day to show the germination progress. He will then fill in a simple data sheet, which answers different questions every day relating to seed condition, and root and leaf growth for every seed.

The photos will be sent to earth as soon as possible where they will be published on a web portal. Participating students will carry out exactly the same experiment on earth at the same time as the astronaut, also taking photos and filling out log sheets. These photos will also be published on the web portal to show the differences between the germination process in Space and on Earth.

### 2. Seedlings

This experiment is also related to plant germination. Sprouts of herbaceous crops (e.g. soybean, broccoli) are widely produced in several countries for food purposes due to the high nutritional value of this product.

The production of sprouts is very fast (5-7 days) and easy; it requires only seeds, water and suitable environmental conditions (e.g. temperature, relative humidity). For these reasons, producing sprouts directly in the ISS may represent an interesting opportunity to offer high-quality fresh food to the astronauts.

The objectives of the experiment are: to evaluate the feasibility of producing vegetable sprouts in space for food purposes and to study the influence of weightlessness on germination, growth and the nutritional quality of sprouts.



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The key questions are:

What is the influence of weightlessness on germination, growth and nutritional quality of sprouts? Is our production system suitable to produce vegetable sprouts in space with an acceptable level of product quality?

### **How is it done?**

The experiment consists of two hermetically sealed plastic bags. Each bag contains two grams of rocket seeds, which are fixed to a paper towel, and a small water container.

The experiment will be carried out during 7 days in orbit. The astronaut will start the germination of the seeds by opening a water container inside the plastic bag. Starting on the first day of germination, a digital photo of the germinating seeds will be taken by the astronaut. This will occur at the same time every day to show the germination progress. He will then fill in a simple data sheet, which answers different questions every day relating to root and leaf growth.

On return to earth the plastic bags and seedlings will be frozen and sent for quality analysis (e.g. vitamin C, carbohydrates, nitrates, antioxidants). The memory card with the images on will also be returned to earth.

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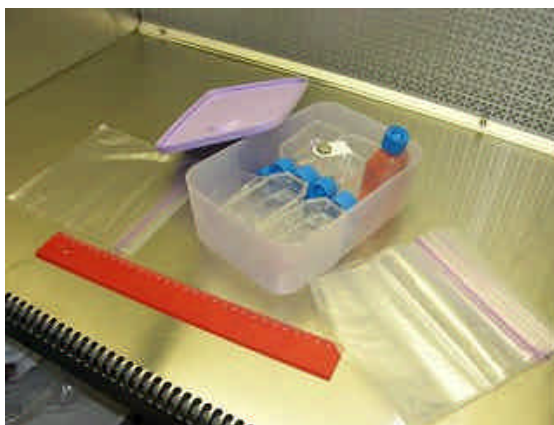
### Fischer Rat Thyroid Low serum 5% (FRTL5)

This experiment is aimed at assessing the effects of the Space environment (microgravity and radiation) on normal in vitro cultures of rat thyroid cells. This unique in vitro test system allows for the use of cells in a quiescent state (non proliferating), which can be kept almost indefinitely without culture medium exchanges or any manipulation.

The cell type chosen are the FRTL5 rat thyroid cell strain, hence the name of the experiment. One of the reasons for choosing these specific thyroid cells is the relevance they have to human physiology and medicine. Thyroid tissue is an ideal target for Space radiation research. The thyroid is a major endocrine gland, central in hormonal regulation in man.

Thyroid tissue has been reported to be strongly resistant to the acute effects of radiation. On the other hand, follow-up studies on human subjects which had been variably but heavily irradiated in Hiroshima, Nagasaki, the Marshall Islands and more recently in Chernobyl, clearly demonstrated a significant long-term sensitivity of thyroid tissue to radiation in terms of development of tumours.

The FRTL5 cells will be used as a biological system to measure radiation and microgravity effects. This experiment should provide further indications that may help in understanding why the sensitivity of the cells to radiation damage is related to their cell cycle and to the kinetics of the radiation. Furthermore it will help to improve our knowledge of the effect of the space environment on the human body, especially with longer-term missions planned in the future (e.g. Mars).



FRTL5 equipment (Image: S. Ambesi)

#### **How is it done?**

The FRTL-5 cells will be placed in culture flasks. Each one is made of optically clear polystyrene with a 75cm<sup>2</sup> available growth area, which has the right surface structure that is specially treated for optimal attachment and growth of cells.

Half of the flasks will contain FRTL-5 cells within a culture that contains a thyroid stimulating hormone. This is a hormone normally secreted by the pituitary gland, which stimulates the thyroid cells to produce thyroxine. The other half will contain FRTL-5 cells in a culture, which does not contain this thyroid stimulating hormone.

The sealed flasks will be accommodated in a zip log bag and all the flasks will be placed in a closed plastic container. The cultures will be kept under a controlled temperature of 37°C by means of an incubator for the duration of the mission.

The experiment set up makes it possible to preserve the cells for a long time both in the proliferate state when being stimulated by thyroid stimulating hormone and in the quiescent state when not. This can be done without any exchange of the culture medium in which the cells are placed and without any manipulation.

The cells will be tested on return to Earth for DNA modifications due to radiation and magnetic fields, and the effect of weightlessness on cell behaviour. In evaluating the response of cells to the thyroid-stimulating hormone, cells will be analysed for indications of mutation and changes in complex cell behaviour such as programmed cell death, and duplication efficiency.

For comparative purposes, these samples will be analysed against samples from a duplicate experiment, which takes place at the same time with the same procedures on Earth.

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## Microbial life in Space: Response to environmental factors in a space vehicle (MICROSPACE)

Microorganisms are well known for their capabilities to withstand extreme environmental conditions such as elevated temperature, high salinity, hydrostatic pressure, and toxic compounds. The exposure to radiations, vacuum, electricity, and magnetic waves has been investigated in the past, but still little information is available about the effects of the space environment on microorganisms.

Different microbial strains will be flown to the ISS in order to study the effect that space radiation and the weightless environmental conditions onboard the ISS have on the cultures. The microbial strains selected for the experiment are non hazardous, of environmental origin, and some of them (*Saccharomyces cerevisiae*, *Escherichia coli*, *Enterococcus faecium*, *Lactobacillus acidophilus*) are commonly hosted by humans.



Hardware set: Sigma centrifuge tubes, cryo-vials, smart button, microbiological cotton, Ziploc bag (Image: F. Cangarella)

This experiment may improve our understanding on the basic biology of microorganisms, particularly on their tolerance to the spacecraft environment and on how the genetic material in the cells can be affected by in-flight space conditions. The experiment can further provide a greater understanding of the spacecraft environment itself.

### How is it done?

Microorganisms will be tested as lyophilised (free-dried) cultures and/or spores in order to evaluate cell number, viability and cell integrity.

The selected strains will be treated as live but non-active cells in order to optimise expenses, experimental work, and to make both storage and transportation easier. Moreover no crew is involved in executing the experiment.

In the ground lab the selected cultures will be cultivated under optimal conditions and stored until the experiment begins. At this stage (T0) lyophilised cultures and/or spores will be prepared, appropriately packed, and shipped; part of these cultures will be collected and analysed (cell number, viability, cell integrity, and genetic pattern) as a T0 control.

Microorganisms will be kept inside 2 ml cryo-vials and then placed inside 80 ml Sigma centrifuge tubes that will be assembled in the lab as sub-packages in Ziploc-like bags and easily transported to the launch site.

A Smartbutton temperature data logger will be added to each pouch. A filter made of microbiological cotton will be inserted inside each Sigma Centrifuge tube.

Three pouches containing identical samples will be assembled so that they are tested at different mission times. At the end of the present mission two pouches will be retrieved by Soyuz and one will remain on the ISS until the next mission.

Once back at the ground lab, quantification of cells will be carried out by means of optical microscopy, whereas the morphological cell structure will be determined via electron microscopy.

As the stress the cells are exposed to during the flight mission may induce modifications in the genome of microorganisms, a further topic of the present experiment is the analysis of total DNA by means of molecular techniques.

A ground reference experiment will be kept in the Laboratory of Agricultural and Environmental Microbiology at the University of Tuscia and analysed following the same protocol, so that it will be possible to compare the response of microorganisms under both space and terrestrial conditions.



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The selected microbial cultures are chosen among genera *Saccharomyces*, *Escherichia*, *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterococcus*, *Streptococcus* and *Thermococcus*, so that the work may be performed with species of different environmental origins and capable of surviving during the mission and under the onboard average environmental conditions. Moreover, further experiments will be carried out using the same bacterial species in a simulator on Earth to investigate the effects of space radiations on bacteria.

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### Vine In Near Orbit (VINO)

Numerous experiments involving the growth of plants and seeds in altered gravity have been conducted in the past. This scientific area has also recently received more attention with the perspectives of very long duration orbital flights, in particular future human exploration of Mars.

Historically, vine growing has always played a vital part of everyday human life. In fact, archaeological findings have managed to date vine growing to as far back as 5600 b.C. It is fascinating if we imagine vines accompanying men and women into the future too.

A new experiment idea involving vines has been conceived and financed by a collaborative effort between different vineyard associations in Tuscany, the Province of Livorno and the company Kayser Italia.

The aim of the VINO experiment is to test the survival and growth in space of tendril grafts from vines coming from Sassicaia vineyards in Tuscany, Italy. Tendrils are twisting, threadlike structures by which a twining plant, such as a grape or cucumber, grasps an object or a plant for support.



VINO Experiment Container (Image: Kayser Italia)

#### **How is it done?**

The vine grafts, will be launched into orbit in a pressure/vacuum proof sealed metallic container (18cm x 16cm x 11cm) to avoid any environment contamination, with the cuts and grafting already executed on ground. Inside the container the grafts will be kept within plastic bags.

Once docked to the ISS the container and samples will be moved to the Russian segment of the ISS where they will remain in a passive state until being transported to earth at the end of the mission.

The experiment container is not fitted with any thermal control; the experiment will be kept at the general temperature conditions of the Soyuz (during launch and orbital flight) and of the ISS. This is between 18°C and 28°C.

The grafts are placed in a plastic bag, where they will remain until the end of the in-orbit section of the experiment.

Once back on the Earth, the tendrils will be implanted, to test their growth. These will be compared to equivalent plants that were treated in a similar fashion in parallel on the ground for reference purposes.

This experiment could provide useful results regarding plant survival in future long duration space flights such as in the human exploration of Mars.

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## Crickets In Space 2 (CRISP-2)

This experiment follows on from a previous experiment, CRISP, performed on the Neurolab STS-90 mission in 1998. Results revealed that the development of gravity-related behaviour in crickets was not affected by weightlessness while the physiology of a specific neuron linked to posture sensitivity was modified.

The question that CRISP-2 will try to answer is whether weightlessness affects the proliferation of neurons if fertilization occurs in space, and what the impact on gravity related behaviour will be.

The experiment uses crickets (*Acheta domestica*) as test specimens, because they possess neurons that can be unequivocally identified in each individual animal. This specific feature allows for the study of the anatomical and physiological characteristics of neurons in the context of their natural environment (the body itself) in a large number of animals.

### How is it done?

The very first step in the formation of neurons and neuronal networks has to occur in the absence of gravity, i.e. fertilization of eggs in weightlessness is mandatory.

The fourteen female crickets used for the CRISP-2 experiment will be inseminated in a ground lab before launch and because they are able to store sperm for weeks, the presence of male crickets for fertilization in-orbit is not required. The fertilization process takes place immediately before female crickets deposit eggs into the ground.



Egg collectors in open and closed configuration (Image: E. Horn)  
The 14 female crickets will be accommodated in the Adult Compartments (CC-AC) of 2 Cricket

Containers (CC). To ensure the fertilization does not take place before being under weightless conditions, the females will not initially have access to the soil-filled Egg Collectors (CC-EC) located in the 2 CC. The Cricket Containers also contain two compartments for hatching larvae (CC-LC).



One of the two Experiment containers, showing the adult compartment (CC-AC), the egg collectors (CC-EC) and hatching larvae compartments (CC-LC) (Image: E. Horn)

Once on-board the ISS, the Egg Collectors will be rotated twice so that the females will have access to and be able to deposit eggs in the soil twice during the mission.

The first set of eggs will have sufficient time for the neural development to be completed in the embryos. The second set of embryos will continue this process after return to earth, i.e. under normal gravity conditions.

Upon return to Earth, the adults and embryos within the CC will be transferred to the ground labs for immediate gravity related-behavioural and neuroanatomical tests.

In the previous CRISP experiment the gravity related behaviour was studied by analysing compensatory head movements that are induced by lateral rolls of the animal. However, due to the delicate structure of the animals, these have proven to be difficult to record. For CRISP-2, this part of the experiment will be replaced by geotaxis tests.

Geotaxis is the orientation with respect to gravity on an inclined surface. The post-flight gravity related behaviour will be analysed by recording



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the walking direction of the crickets up a slope for different inclination angles of the slope.

The neuroanatomical tests that will be conducted include analyses of the size of cell bodies of neurons and the pattern of dendrites in embryos and hatched larvae. Dendrites are the short, highly-branched fibres that carry signals toward the cell body of a neuron.

The females will be allowed to deposit eggs for a third time under normal gravity conditions. The success of this period of egg deposition will be determined 15 days later by counting the hatching larvae and by executing further neuroanatomical and behavioural (geotaxis) tests.

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