Using Biologicals in SSEP Experiments: Dormant Forms, Fixatives, and Growth Inhibitors

Harri Vanhala, Ph.D., NCESSE Adjunct Space Science Researcher

Revised: September 7, 2019

1. Introduction

The SSEP missions to the International Space Station (ISS) are a great opportunity for student teams to conduct biological experiments in microgravity, given the flexibility of experiment design allowed by the mini-laboratory – the Fluids Mixing Enclosure (FME). Since the FME can have up to three separate experiment volumes, there are numerous ways to design a successful experiment that keeps samples (fluids and solids) separated until the specific times when the experiment calls for them to be mixed. However, there are several considerations regarding the timeline of the mission that the student teams need to take into account when designing biological experiments. Of special interest are:

a) The time it takes to transport the FME from the student team after loading until it arrives at the ISS; this is expected to take approximately 3 weeks;
b) The duration of the experiment aboard the ISS; while the FME is nominally scheduled to be aboard the ISS for approximately 4-6 weeks, a student team can request their experiment be activated on a specific Crew Interaction Day during this period;
c) The time it takes to transport the FME from the ISS back to the student team; it is expected to take 1-3 days from the time the FME is transferred from the ISS to the ferry spacecraft, the ferry spacecraft touches down on Earth, and the payload arrives in Houston. If the FME is shipped to the student team (that is, if a representative of the team does not pick up the FME in Houston in person), the shipping time must be taken into account, as well.

See the Mission 14 to ISS Mini-Laboratory Operation page (http://ssep.ncesse.org/?p=24227) for more details on the way the FME can be configured, and for information on the critical timeline for operation of the mini-laboratory during the mission.

To address Points a, b and c described above requires careful experiment design. If the experiment is biological, it is highly likely that the organisms to be investigated in the FME will need to be obtained and loaded in a dormant form and activated in orbit, by for example mixing freeze-dried organisms with a liquid growth medium, or mixing dry seeds with water. It is hard to imagine that active organisms loaded in the FME will survive for several weeks before reaching orbit.

The student team has control of Point b listed above – the duration of the experiment aboard the ISS – given they can decide on the best Crew Interaction Day to activate and deactivate the experiment during the flight.

Regarding Point c, you might not want the experiment to proceed once it leaves orbit, because the FME will then experience possibly up to 4-5 days of gravity here on Earth before being returned to the student team (including time for shipping from Houston back to your community). So you might want to either stop or slow down the growth of the organisms and preserve them for analysis by mixing in a "fixative" or a "growth inhibitor" before the experiment leaves the ISS.

These aspects of experiment design are discussed in greater detail in the following sections.

2. Using Dormant Forms of Organisms

Since it will take several weeks for the FME to be transported to the ISS, it is likely that active organisms loaded into the mini-laboratory by the student team will reproduce and grow to the point where they will run out of resources, perish, and decay before the mini-laboratory reaches the microgravity environment. For many biological experiments, such as seed germination studies, this is not a significant concern, since seeds are naturally dormant, and the experiment can be activated in orbit simply by bringing dry seeds contained in one experiment volume of the FME into contact with water contained in another volume. For other experiments, such as bacteria and cell studies, and aquatic life experiments, the long transportation time is likely to affect the experiment design. Some types of bacteria form spores, some aquatic life species produce eggs that are dormant until they are revived when conditions become favorable for them to develop, and other kinds of bacteria, as well as cell cultures, tissue samples and some simple multicellular organisms, can be prepared for long-term storage in a variety of ways, such as freeze-drying. The basic approach to experiment design can therefore be the same as for seed studies: dormant forms of organisms can be placed in one experiment volume, an activating solution in another, and the two samples are brought together in orbit to start the experiment. There are numerous ways to prepare organisms for the long transportation time, and numerous activating solutions that can be used depending on the exact sample to be activated. As a result, it is not possible to list all available methods of preservation and activation here. Instead, it is highly recommended for the student teams planning to conduct biological experiments to make connections with local professional scientists conducting research in the topics the teams are interested in exploring. The researchers can provide invaluable guidance in helping the team design an experiment that employs the ideal dormant forms of the organisms and the best way to activate the organisms once the experiment reaches the ISS. Local professionals may also have access to appropriate samples in their own stock without the students having to obtain new materials from commercial vendors, providing the student teams easy access to ready-to-use samples. The researchers can also help the student team throughout the experiment process. See Section 5 below for more information on making connections to local researchers. While this is the recommended method of working with biologicals, if the student teams want to obtain the biological sample materials from commercial vendors on their own, Section 6 includes more information on a few possible vendors to consider.

3. Fixatives and Growth Inhibitors

While the transportation time for the FME from the ISS to the student teams is fairly short (a couple of days), for some experiments it may be too long for the effect of microgravity to remain detectable after the samples are returned to normal gravity conditions on Earth. In other words, reintroduction to gravity could ruin the experiment. Therefore, the student team may want to terminate the experiment at a certain point before leaving ISS. This is a common aspect of biological experiments, since there are many instances where a researcher may want an experiment to be conducted over a specific number of days and then be terminated. If the experiment is allowed to continue, the results may not be as useful. To terminate the experiment, scientists can use *fixatives* to kill and preserve the organisms or *growth inhibitors* to slow down the growth of the organisms.

Fixatives are useful for preserving biological samples because they stop biochemical reactions, kill the organisms, and preserve the cells and/or tissue as close to their natural state as possible. They also may change the samples on a molecular level to increase their stability, which helps preserve the shape and structure of the samples for analysis. The downside of using fixatives is that to work, they also alter the sample to some degree, and so they may introduce structural changes that could be interpreted as being natural. The effect of these kinds of artificial features – artifacts – can be reduced by carefully choosing the right kind of fixative and by limiting the time the sample is exposed to the fixative. Another downside of using fixatives is that they can be toxic; they are typically categorized as hazardous materials and need to be handled using extensive safety precautions.

An alternative to using fixatives is to employ biological growth inhibitors—antibiotics. They can be used to halt cellular activity without actually fixing (killing) the cells. The benefit of using this approach is that there is no need to store the samples in a fixative solution for a long period of time. Growth inhibitors also do not typically kill the organisms, and so the samples could be cultured in a laboratory after landing. Also, antibiotics are typically less hazardous materials than fixatives, though appropriate safety procedures must be followed when handling these samples, as well. However, there are downsides to using antibiotics, such as the possibility of causing changes in the structure of the cells in the sample.

An example of the way that fixatives and growth inhibitors can be used in an experiment on a SSEP mission to the International Space Station could be a dormant biological sample (containing bacteria spores, for example) loaded into one experiment volume of the FME. The sample remains dormant until it is activated aboard the ISS by exposing it to an activating/nutrient solution contained in another experiment volume of the mini-laboratory. After a desired time period, the biological sample could be fixed, or its growth slowed down, by mixing in a fixative or a growth inhibitor contained in the third experiment volume of the FME.

There are numerous ways to terminate a biological experiment using a variety of fixatives or growth inhibitors, and it is not possible to list all methods and chemicals here. Instead, it is highly recommended for the student teams planning to conduct biological experiments to make connections with local professional scientists conducting research in the topics the teams are interested in exploring. The researchers can help the team decide whether their experiment needs to be terminated before landing, and what are the best ways to use fixatives or growth inhibitors

for this purpose. Local professionals may also have access to appropriate materials in their laboratory without the students having to obtain these potentially hazardous materials from a commercial vendor, providing the student teams easy access to fixatives and growth inhibitors ready for use in an appropriate handling environment. The researchers can also help the student team throughout the experiment process, including formulating best strategies for analyzing the samples after the flight. See Section 5 below for more information on making connections to local researchers.

4. Examples of Fixatives and Growth Inhibitors: Solutions Used During SSEP Flight Opportunities

Students can choose to include in their experiment design, fixatives and growth inhibitors that best meet their experiment objectives. If a student team wants to obtain or prepare their own fixatives or growth inhibitors for use in their experiment, there are numerous commercial vendors (a few examples listed in Section 6 below) from which appropriate materials can be purchased. Note, however, the restrictions on allowable samples for SSEP flight opportunities to ISS:

a. No radioactive materials, perfumes, hydrofluoric acid, magnets, cadmium, beryllium, or acetone can be flown.
b. Teams may not propose to fly technology. This includes batteries, lighting, and any device associated with electrical circuits and/or mechanical systems.
c. If a sample is listed in the **NanoRacks List of Problematic Samples** document (found in the Document Library), which details samples that may adversely interact with the mini-lab's silicone tube and/or end caps, it is imperative that students follow the guidelines on the Mission 14 to ISS Mini-lab Operations page (http://ssep.ncesse.org/?p=24227) before proposing any samples from the **NanoRacks List of Problematic Samples**.
d. NanoRacks and NASA reserve the right to refuse other fluids/solids based on hazard level. All student teams are advised to consider carefully the level of hazard posed by the samples they are planning to use. Examples of hazardous samples, include: fluids/solids that are hazardous enough that there is a concern that the student team is even handling these substances; fluids/solids that when mixed can result in excess hear and/or pressure inside the tube, leading to loss of containment, and fluids/solids where there is evidence of excess heat – even chemically generated light – that could adversely impact other FMEs that share the payload box on ISS; biological with a designated BioSafety Level (BSL) of 2 or higher. It imperative that students follow the guidelines on the Mission 14 to ISS Mini-lab Operations page (http://ssep.ncesse.org/?p=24227) before proposing any samples that could be considered hazardous under these guidelines.

This is in contrast with the previous SSEP flight opportunities on the Space Shuttle, where only a few fixatives or growth inhibitors were allowed and only those on a **Master List of Experiment Samples**. (You can find the Master List in the Document Library at the SSEP website.)

While student teams are no longer required to use only fixatives and inhibitors on the Master List, we provide a discussion below of those that appeared on the List in order to prompt thinking into what types would work best for different experiments.

One of the most common fixatives used today is formalin, and the recommended solution for the SSEP flight opportunity on the STS-135 Shuttle flight was:

> 10% Neutral Buffered Formalin (NBF): approx. 3.7% formaldehyde in phosphate buffered saline

Formalin works well as a general fixative and can be used for a variety of biological experiments. If a team is considering using formalin, it is advised to keep in mind that formalin is a hazardous material. The team needs to be certain that it needs to use this fixative, and it must carefully follow all safety guidelines. Also, long-term exposure to formalin may adversely affect the biological sample, so the team will want to make sure the exposure time is sufficiently short for the samples to remain useful.

Another previously allowed fixative was RNAlater, which is a commercial vendor kit designed for a specific fixing purpose: to preserve RNA for analysis. Because of this specificity, it may not be useful for most SSEP experiments, but for those interested in investigating RNA, it is a useful alternative to formalin, given that it is non-toxic and therefore safer to handle.

Antibiotics that were used as growth inhibitors during the Shuttle Flights included: puromycin, and a combination of rifampicin and cephalexin. Examples of possible concentrations that could be used for different kinds of organisms were:

Prokaryotes:

Puromycin: 100-200 micrograms/milliliter (*e.g.,* for *Bacillus subtilis*)

Rifampicin: 150 micrograms/milliliter mixed with cephalexin: 10 micrograms/milliliter (*e.g.,* for *E. coli*)

Eukaryotes:

Puromycin: 0.5 micrograms/milliliter (*e.g.,* for murine cells)

As explained above, the student teams are allowed to use different types of fixatives and antibiotics for their experiment design, and the descriptions above are just suggestions, not requirements. For example, ethanol, which was not allowed on the SSEP flight opportunities aboard the Space Shuttle, is considered a good general-purpose fixative and is less toxic than formalin. However, ethanol is listed on the **NanoRacks List of Problematic Samples** document, and whether it would be allowed to fly to ISS would need to be assessed with NCESSE based on proposed concentration.

5. Collaborating with Local Universities and Research Laboratories

It is highly recommended for the student teams interested in designing biological experiments to make connections with local professional scientists conducting research in the topics the teams are interested in exploring. The researchers can provide invaluable guidance in all aspects of experiment design to address the considerations discussed in this document. They can help the student teams identify the best dormant organisms to use in the experiments, the most reliable activation methods once the experiments are to be started in orbit, the best growth media for the

organisms once they're active, and the best ways to terminate the experiments with the use of fixatives or growth inhibitors, if necessary. Finally, the researchers are able to help the student team develop appropriate analysis methods to address the questions the students want to answer with their experiment.

Local researchers may also have access to appropriate materials in their laboratories without the students having to obtain them from commercial vendors. This is important, since as part of their proposals, the student teams need to demonstrate that they have access to all the samples to be used in their experiments, and collaborating with local researchers who have access to the necessary materials in their laboratories will address this aspect of the proposal easily.

While local professional scientists are recommended as science advisors, the student team preparing a proposal must remember that adults – whether parents, teachers, or researchers – cannot be the main force behind the proposal. The SSEP experiment is intended to be a student-driven effort, with the students designing the experiment, writing the proposal, and conducting the actual experiment, with adults offering guidance, mentoring and supervision, as best meets the needs of the student team and their experiment. Professional scientists in universities and research laboratories are ideal in this regard, since they are always interested in mentoring the next generation of scientists in their fields of interest.

The easiest way to start creating pathways to a collaboration with local professional scientists is to contact the chair of a biology department in a nearby university or research laboratory or at a research hospital. These key individuals will be able to identify scientists on their staff that are most suitable for acting as advisors to the student teams. In fact, many student teams that have participated in previous SSEP flight opportunities have started their collaboration with local professional researchers this way, and the advice provided by the scientists has been invaluable in making the student experiments successful. Indeed, this is a great pathway for all student teams to build connections to their local professional research community. If you need assistance making connections to suitable research advisors in your community, NCESSE can help!

6. Commercial Vendors of Biological Samples

If student teams want to obtain and prepare their own biological samples without collaborating with professional researchers in their area, there are numerous vendors that provide biologicals for research. In many cases, school systems may have accounts set up with particular vendors already, so the teams are advised to find out whether their school has a preferred vendor for biologicals. If there is no preferred vendor, the teams interested in obtaining their own biologicals are advised to contact possible vendors as soon as possible to see what steps may be required for the team to be able to receive biological shipments. Below is a list of vendors that provide biological supplies – dormant organisms, cultures, growth media, fixatives, and growth inhibitors – to researchers in a variety of settings. The list below is by no means complete, and the teams are not required to use any of these vendors as their source. The list of a few possible vendors for biological samples includes:

ATCC: http://www.atcc.org/
Carolina Biological Supply Company: http://www.carolina.com/
Edvotek: http://www.edvotek.com/
Thermo Fisher Scientific: https://www.thermofisher.com

Fisher Scientific: http://www.fishersci.com/
RNALater: http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/rnalater.html
Hardy Diagnostics: http://www.hardydiagnostics.com/
Sigma-Aldrich: http://www.sigmaaldrich.com/

These companies typically have large stocks of biologicals, both as active cultures and in dormant forms. For example, a search for freeze-dried products at ATCC provided over 1,000 results.

An important note: commercial vendors are able to make many biological samples available only to research institutions, and not to schools. This is another important reason to collaborate with researchers in your area.

7. Describing the Use of Biological Samples in Your SSEP Proposal

As discussed in the previous sections, there are many aspects of experiment design the student team needs to consider carefully when proposing a biological experiment to fly on a SSEP mission to the International Space Station. It is crucial for the team to describe how they are planning to address all these aspects in their experiment design. For example, how are the biological samples preserved during transport of the FME from the student team to the ISS? How are the (possibly) dormant organisms activated once the experiment is to be started in orbit? How is the experiment terminated, if necessary? Does the student team have access to all the samples they need to conduct the experiment?

Besides just explaining how the team is planning to address these questions in their experiment, the proposal needs to demonstrate that the plan is likely to work. For example, if the team plans to use freeze-dried organisms that are activated in orbit using a specific activating/nutrient solution, it is highly advisable for the team to test the procedure before writing their proposal by exposing the freeze-dried organisms to the proposed activating solution and describing the results of the test in their proposal. This will convince the reviewers that the proposed activation process is likely to work when the experiment is activated in orbit. The same is true for terminating the experiment: it would be highly advisable for the student team to show that their fixative or growth inhibitor works for terminating the experiment at a desired point. The team does not have to do a complete dry run of the experiment using actual flight hardware for the proposal, just demonstrate that the critical points of the experiment are likely to work as described. Note that the process of testing the various stages of the experiment and explaining the results in a proposal is what professional scientists do when they prepare their research proposals. This is another example of the way the SSEP proposal process follows the guidelines used by professional scientists.