

Phobos LIFE (Living Interplanetary Flight Experiment)

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Abstract

The Planetary Society's Phobos Living Interplanetary Flight Experiment (Phobos LIFE) flew in the sample return capsule of the Russian Federal Space Agency's Phobos Grunt mission and was to have been a test of one aspect of the hypothesis that life can move between nearby planets within ejected rocks. Although the Phobos Grunt mission failed, we present here the scientific and engineering design and motivation of the Phobos LIFE experiment to assist with the scientific and engineering design of similar future experiments. Phobos LIFE flew selected organisms in a simulated meteoroid. The 34-month voyage would have been the first such test to occur in the high-radiation environment outside the protection of Earth's magnetosphere for more than a few days. The patented Phobos LIFE "biomodule" is an 88 g cylinder consisting of a titanium outer shell, several types of redundant seals, and 31 individual Delrin sample containers. Phobos LIFE contained 10 different organisms, representing all three domains of life, and one soil sample. The organisms are all very well characterized, most with sequenced genomes. Most are extremophiles, and most have flown in low Earth orbit. Upon return from space, the health and characteristics of organisms were to have been compared with controls that remained on Earth and have not yet been opened. Key Words: Phobos LIFE—Spaceflight experiments—Transpermia—Panspermia—Lithopanspermia. *Astrobiology* 19, 1177–1185.

1. Introduction

LIFE EITHER EMERGED *in situ* from prebiotic matter during Earth's Archean eon, or Earth was seeded by life that arrived here from another planet or moon. The latter hypothesis represents an idea dating back to antiquity (Sider, 1981) and experiencing a resurgence in thought more than a hundred years ago (Richter, 1865; Thomson, 1894; Arrhenius, 1903). Often, the term "panspermia" has been used in connection with the seeding scenario, but this term connotes the radiation of life throughout the Cosmos from a single, distant point. Given the prospect that short-range

spread of life between planets and moons of the same star system must be easier than seeding over interstellar and intergalactic distances, other terms may be employed to connote the spread of life by meteoroids within our solar system. One such term is "transpermia," coined by Oliver Morton, circa 2000 (Morton, 2013, personal communication to D. Warmflash); we have used it in previous publications focusing particularly on transfer of life from Mars to Earth (Warmflash *et al.*, 2007). Given the proximity of the planets of the inner Solar System, the low gravity well of Mars, and the fact that materials ejected into space move more easily from outer to inner orbits about the Sun (Kirschvink and

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Weiss, 2002), Mars-to-Earth is the life transfer scenario that is discussed most frequently (Melosh, 1985, 1988).

Whether terrestrial life could have originated on Mars depends on the ability of microorganisms to survive the voyage. The notion that loose microorganisms could escape a planet's gravity well and survive radiation and vacuum and entry through a planetary atmosphere appears tenuous. However, the concept that microorganisms could be ejected within rocks and travel successfully to another planet within those meteoroids is more plausible. Approximately one ton of martian rock ejected via major impact events arrives on Earth each year in the form of meteorites (Gladman, 1997). Many tens of meteorites have been identified as having originated in the martian crust (Nyquist *et al.*, 2001; Meyer, 2012); this identification was confirmed by argon isotope fractionation studies using the Sample Analysis at Mars (SAM) instrument on NASA's Curiosity rover (Atreya *et al.*, 2013). These represent only a tiny sampling of transferred martian rocks.

Although most of the interplanetary material that arrives on Earth has spent several million years in space, it is estimated that one out of 10^7 Earth-impacting martian rocks has made the interplanetary journey in less than a year, and that every million years, approximately 10 rocks larger than 100 g are transferred from Mars to Earth in only 2–3 years (Gladman, 1997). If present within rock on Mars, microorganisms akin to those on Earth could remain viable when challenged by the pressures and heating of impact events that eject such rock into space (Mastrapa *et al.*, 2001).

Additionally, studies of magnetic features of martian meteorites and gases trapped within suggest that interiors of such rocks are not heated to temperatures high enough to kill microorganisms during entry through Earth's atmosphere (Weiss *et al.*, 2000, 2002; Artemieva and Ivanov, 2004; Shuster and Weiss, 2005).

Whether survival of metabolically active microbial species, or dormant spores, during the interplanetary transfer phase itself would be sufficient to allow for planetary seeding is unknown. Previously, microbial survival in space has been investigated, for periods of up to 6 years, albeit in low Earth orbit (LEO) (Horneck, 1993; Horneck *et al.*, 1974, 1994, 2010; Reitz *et al.*, 1995; Rettberg *et al.*, 2002; Mancinelli, 2015) where high-energy radiation exposure is relatively low. Microbial survival also has been studied outside Earth's magnetosphere, and thus in the interplanetary radiation environment, but for relatively short durations (several days) (Buecker *et al.*, 1973, 1975; Buecker, 1974; Horneck *et al.*, 1974; Buecker and Horneck, 1975; Graul *et al.*, 1975; Facius *et al.*, 1978, 1979). The aforementioned studies, and others conducted subsequently in LEO, have demonstrated survivability of various archaea and bacteria, along with plant seeds and animals (Reitz *et al.*, 1995; Rettberg *et al.*, 2002; Jönsson *et al.*, 2008; Horneck *et al.*, 2010).

To test whether life-forms can survive the transit phase of the swift transfer scenario that occurs among a small fraction of martian ejecta, we prepared the Phobos Living Interplanetary Flight Experiment (Phobos LIFE). To take advantage of the 34 months of round-trip transit time through the space between Earth and Mars, the experiment was carried within the Russian Federal Space Agency's Phobos Sample Return spacecraft (also known as Phobos Grunt). Unfortunately, the mission failed soon after launch. We felt it was still important to document through this paper

the motivation for Phobos LIFE, the science underpinning it, the technical approach and the engineering designs used. Designers of future experiments can use this information as background to test aspects of transfer of life between nearby planets and moons.

2. Science Experiment

Phobos LIFE carried 10 organisms, representing all three domains of life (Bacteria, Eukaryota, and Archaea) and a soil colony sample on the Phobos Grunt Sample Return flight, launched in November 2011. Spending most of the 34-month mission time in deep space, and thus outside Earth's magnetosphere, Phobos Sample Return's basketball-sized return capsule would have functioned as an artificial meteoroid of sorts. The organisms flew in dried, "dormant"-like states inside the 88 g, patented Phobos LIFE "biomodule" (Fraze and Friedman, 2010) (Fig. 1). Since the Grunt return capsule was designed to return to Earth, the passive biomodule would have allowed for the recovery of the samples after nearly 3 years in interplanetary space. The experiment was designed to simulate weightless travel of organisms through deep space inside a meteoroid, thus protected from exposure to vacuum and with some radiation shielding, blocking all the solar ultraviolet radiation, but not enough shielding to stop much of the high-energy radiation encountered in deep space.

Regardless of who originally provided them (detailed below), all samples were shipped to the American Type Culture Collection (ATCC) facilities in Manassas, Virginia, United States, where they were sealed in individual tubes under an argon atmosphere and loaded into the Phobos LIFE biomodule. Argon was used because it is an inert gas that will not interact with the organisms. Under natural conditions on Earth, oxygen interacts with the tissues of desiccated organisms and gives rise to accumulated damage to cell components (França *et al.*, 2007), eventually rendering the organisms nonviable, if total damage exceeds the repair capacity at rehydration. Under interplanetary transport, oxygen will not be present, so argon will provide a more representative condition for this experiment.

Upon returning from space, the samples were to be distributed among the team of investigators who had provided the samples for comparison with preflight phenotypic, genotypic, and other characteristics. Investigators would have looked for basic organism survivability as a result of the deep space voyage and also would have conducted more detailed analyses. Three Earth control biomodules were loaded with organisms from the same batch at the same time that the flight biomodule was loaded and sealed. Sealing occurred in June 2009 in preparation for the initial Phobos Sample Return mission launch date, but the mission was delayed until the launch window had passed, moving launch to 2011. One of the three control biomodules was to have been opened and organism samples analyzed shortly after the time of launch in order to determine the effects of loading and sitting on Earth for more than 2 years. A second control module was to be opened concurrently with the flight biomodule when it returned from space, and a third control was kept as backup. The spacecraft's dangerous situation was recognized soon after launch, and the decision was made to leave the control biomodules sealed.



FIG. 1. Sealed Phobos LIFE biomodule. Hand for scale.

3. The Phobos Life Biomodule

3.1. Requirements

The Phobos Sample Return project required the Phobos LIFE biomodule to be (1) less than 100 g; (2) completely passive; (3) able to survive the equivalent of a 4000g (where 1g is the acceleration of gravity at Earth's surface) impact without loss of structural integrity. Phobos LIFE science requirements included (1) the ability to carry small, single-strain biological samples; (2) the ability to carry a single larger soil sample; (3) practical containers allowing effective handling, loading, and unloading; (4) the ability to seal organisms under argon; (5) redundant seals.

3.2. Design details

To meet the requirements, we created a container (Fig. 2) that uses webs and pockets to provide strength at low mass, and a strong, padded, multiply sealed design to provide structural integrity, with loads up to 4000g. Details of the biomodule's patented (Fraze and Friedman, 2010) design include the following:

The outer housing is titanium, which is both strong and lightweight. The shell is machined with "pockets" which reduce weight but do not diminish strength. The inner carrier is the polymer material Delrin, with an excellent history of space travel and many appealing properties, including ease of machining.

There are 30 Delrin tubes, each 3 mm in diameter, which hold the microbe samples. Unique identifying labels were placed on the top and bottom of each sample tube. Additionally, there is a central Delrin container for the soil sample.

There are several sealing mechanisms in the design. First, the lids seal to the Delrin tubes both through friction fit and

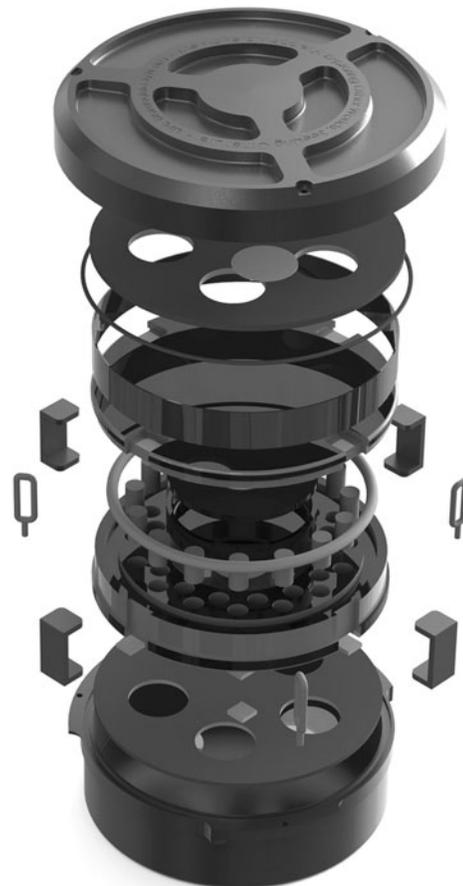


FIG. 2. Exploded view of Phobos LIFE biomodule showing components.



FIG. 3. Heat sealing a sample tube inside argon-filled glove box.

by welding the lids to the tubes using a custom device (Fig. 3). Additionally, the top half of the Delrin carrier presses on the sealed tubes and further prevents the lids from separating during flight.

Next, there is a silicone O-ring sandwiched between the two halves of the carrier external to the sample tubes; this polymer seal acts as a secondary seal (Fig. 4). The Delrin carrier is contained by four titanium clips which keep the two halves of the carrier under sealing pressure. These clips are also retained by Kapton tape circumferentially. The

carrier has polymer pads above and below it to mitigate launch and landing shock.

The sealed and wrapped assembly is placed into the bottom half of the titanium housing; and another seal, made of indium wire, is placed in a groove between the top and bottom housings. The top is turned, engaging three integral locking lugs, which are then safety wired in place to prevent the top from backing out. The metallic indium seal is permanently crushed to seal the housing. Thus, there are several independent seals.

3.3. Temperature and radiation detectors

In addition to the structural components, seals, pads, and bio-load, the biomodule also includes passive temperature and radiation detectors. The radiation detectors (Fig. 5) consist of eight thermoluminescence detectors (TLDs): four each of the types TLD-600 (${}^6\text{LiF:Mg,Ti}$) and TLD-700 (${}^7\text{LiF:Mg,Ti}$). They measure the absorbed dose due to ionizing radiation. Following the mission, they would have been taken into the laboratory for evaluation and determination of the doses of ionizing radiation. The same types of TLDs have been used in the framework of the MATROSHKA experiment on board the International Space Station (Reitz *et al.*, 2009; Berger *et al.*, 2012), as well as in the framework of the DOSIS project for radiation dosimetry on EXPOSE-E (Berger *et al.*, 2013).

The temperature sensors (Fig. 5) were designed to determine the highest temperature incurred. For each biomodule, there were eight circular stickers (six distributed inside and two on the outside), each of which had five temperature “dots.” These dots change color if a particular temperature

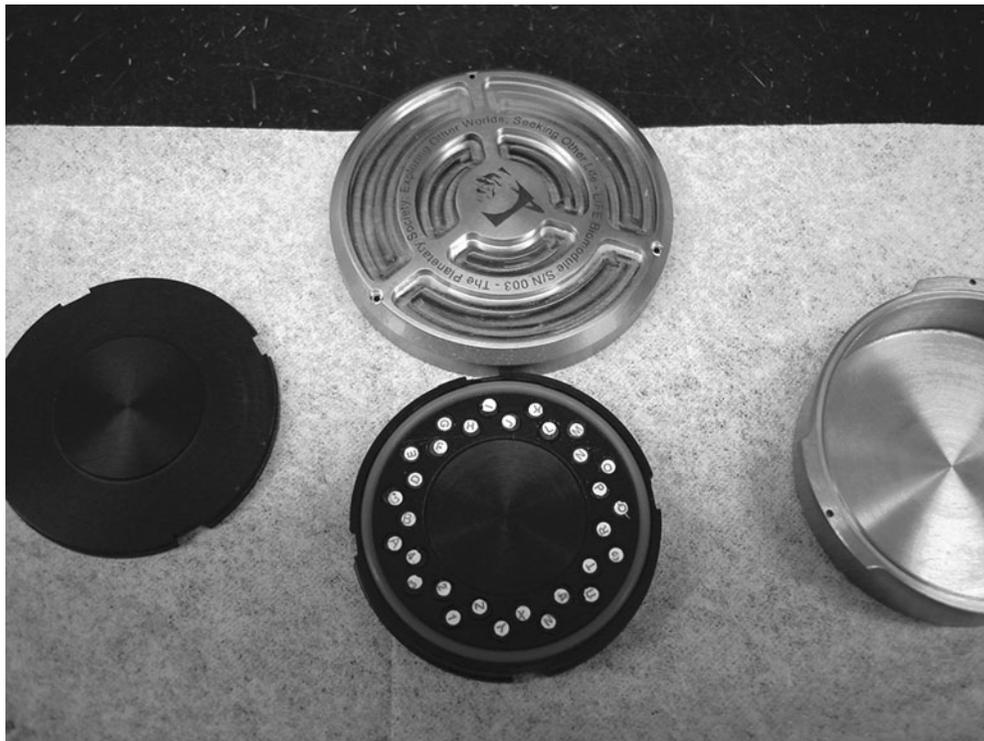


FIG. 4. Lower half of Delrin carrier (center) loaded with labeled sample tubes and central soil container. Note silicone O-ring. Also, top of carrier (left), titanium lid (top), and titanium bottom (right).

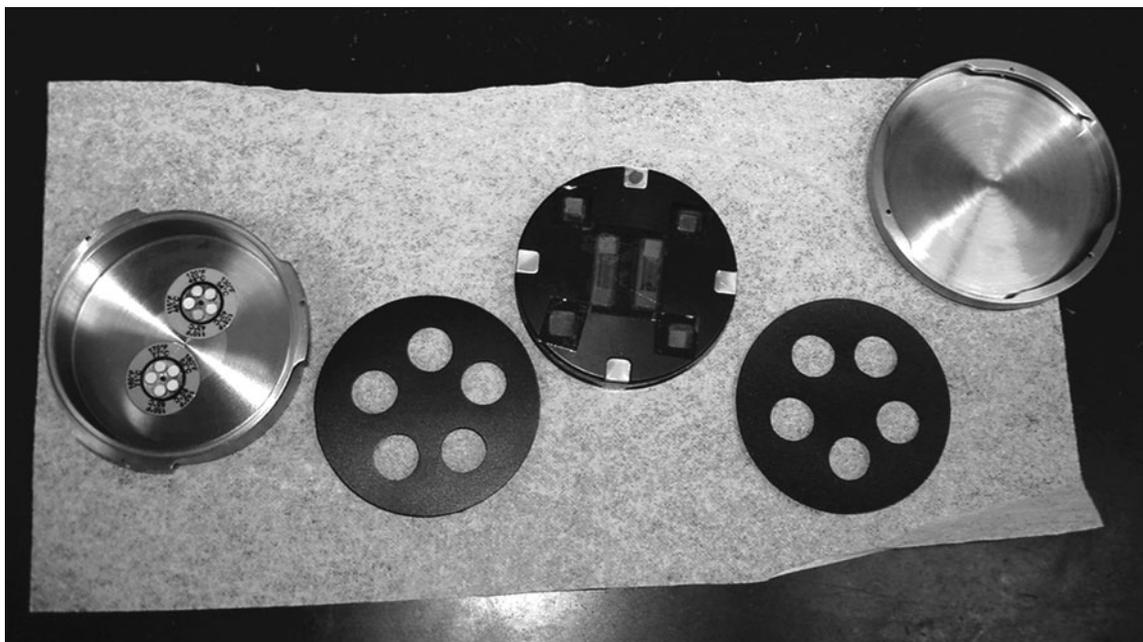


FIG. 5. Biomodule during assembly. Left to right: Titanium bottom with two temperature sensors, shock pad, bottom of loaded carrier including TLD radiation detectors (small white squares under Kapton tape), shock pad, and titanium lid.

is exceeded. Though providing limited information, these dots probably would have revealed the presence of any large thermal spikes penetrating the biomodules during the trip. Knowing this, in turn, would have influenced the interpretation of the biological sample analysis after return of the probe. The temperature stickers come in two temperature ranges giving a total range of 41–82°C (though we did not anticipate temperatures anywhere near the upper end of that range), and they were distributed through various locations in and on the biomodule. Additionally, one of the organisms flying was an extreme thermophile, *Pyrococcus furiosus*. Much of the heat resistance of this organism may depend on active processes, such as production of solutes that protect cellular proteins from denaturation. Such solutes might have been present at some level within the dormant samples of the LIFE biomodule but generally can be expected to protect only active *P. furiosus* cells (Shockley *et al.*, 2003). Nevertheless, if only *P. furiosus* and the spore-forming bacteria (*i.e.*, *Bacillus subtilis* and *Bacillus safensis*, which also are resistant to high temperatures) had survived, while all other organisms had not, this would have constituted an additional datum suggesting thermal heating far beyond what had been anticipated.

3.4. Testing

Many tests were carried out on engineering models of the flight biomodule identical to the flight biomodule. These included vibration tests to simulate launch vibrations, carried out at California Polytechnic State University San Luis Obispo, and impact tests, carried out at Stellar Exploration, Inc. For both tests, each of the 30 sample tubes and 1 colony sample container were filled with a fluorescent liquid. The actual flight samples are dry, but we used liquid as an extra challenging test to make sure that we could identify any

leaks. The entire biomodule was sealed identically to the flight biomodule.

Vibration tests showed that the biomodule and all its components worked perfectly with no leaks, despite being subjected to vibrations beyond those that would be encountered during launch.

An air cannon was used to launch biomodules at high speeds for impact testing. Speeds and impact materials were adjusted to simulate, and exceed, parameters provided by the project, equivalent to about 4000g. In multiple impacts, no leaks in any seals occurred. A heat sealing mechanism was employed for the sample tubes. To make sure the heat sealing process was not detrimental to a biological payload, a series of tests was performed both with temperature sensors and with actual biological materials. The biological materials showed no ill effects, and the temperature sensors indicated minimal heating occurred in the tubes as a result of the heat sealing on the outside.

4. The Organisms

The organisms that flew in Phobos LIFE, and their sources, are detailed in Table 1. This set includes species from the three domains of life. There are two strains of *Bacillus subtilis*, both of which have a long history in spaceflight research (Horneck *et al.*, 2010). The organisms used in the 30 sample tubes meet the following criteria determined by the Phobos LIFE science team:

- There are representatives of all three domains of life (Bacteria, Archaea, and Eukaryota).
- All are nonpathogenic to humans and animals.
- Most have been flown previously in LEO.
- All are very well characterized, most with sequenced genomes.
- Most are extremophiles or extremely robust.

TABLE 1. PHOBOS LIFE ORGANISMS

Organism	ATCC reference number	Type of sample	Form	Organism provided by
<i>Bacillus safensis</i> f036b	ATCC [®] BAA-1126 [™]	Bacteria	Freeze-dried	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
<i>Bacillus subtilis</i> 168	ATCC [®] 23857 [™]	Bacteria	Freeze-dried (ATCC) and air-dried spores (DLR)	ATCC (1 tube), Dr. Tim Lilburn <i>et al.</i> , USA, and DLR (2 tubes), Dr. Petra Rettberg <i>et al.</i> , Germany
<i>Bacillus subtilis</i> MW01	No DLR ref number (Wassmann <i>et al.</i> , 2010, 2011)	Bacteria	Air-dried spores	DLR, Dr. Petra Rettberg <i>et al.</i> , Germany
<i>Deinococcus radiodurans</i> R1	ATCC [®] BAA-816 [™]	Bacteria	Freeze-dried	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
<i>Saccharomyces cerevisiae</i> strain W303	ATCC [®] 200060 [™]	Yeast	Freeze-dried	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
<i>Arabidopsis thaliana</i>		Seeds	Seeds	Dr. David Warmflash, USA; original source: Arabidopsis Biological Resource Center (ABRC), Ohio State University
<i>Milnesium tardigradum</i>		Animals	Air-dried	Kristianstad University, Dr. Ingemar Jönsson, Sweden
<i>Richtersius coronifer</i>		Animals	Air-dried	Kristianstad University, Dr. Ingemar Jönsson, Sweden
<i>Echiniscus testudo</i>		Animals	Air-dried	Kristianstad University, Dr. Ingemar Jönsson, Sweden
<i>Haloarcula marismortui</i>	ATCC [®] 43049 [™]	Archaea	Air-dried with salt	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
<i>Pyrococcus furiosus</i>	ATCC [®] 43587 [™] (DSM-3638)	Archaea	Freeze-dried	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
<i>Methanothermobacter wolfeii</i>	ATCC [®] 43096 [™]	Archaea	Air-dried	ATCC, Dr. Tim Lilburn <i>et al.</i> , USA
Soil microbial community		Arid soil	Air-dried	Moscow State University, Dr. Elena Vorobyova, Russia

- All are in dormant states (inactive, nonreplicating) while packaged within the biomodules: some are dormant as endospores, some are seeds (*Arabidopsis thaliana*), while others have been otherwise desiccated in the laboratory.
- Each organism sample consisted of a distinct species, or strain, stored in three tubes. The exceptions were the tardigrades; these also occupied three tubes, but each tube contained a mixture of three tardigrade species.

The soil sample in the center of the biomodule (arid soil from the Negev Desert, Israel) contained a native soil microbial community, based on indications that colonies of different kinds of organisms may have enhanced survival abilities *in situ* (Vorobyova *et al.*, 1997; Soina and Vorobyova, 2004; Gilichinsky *et al.*, 2007; Tarlera *et al.*, 2008, Shade *et al.*, 2013). Air-dried bacteria and archaea were dried in a sterile environment in the Delrin sample tubes. Freeze-dried archaea and bacteria were taken directly from storage tubes. The freeze-dried pellets of cells were broken

up and loaded into the Delrin sample tube (Fig. 6), and the amount added was weighed.

5. Shuttle LIFE

A related experiment, Shuttle LIFE, flew on space shuttle mission STS-134 in May 2011. A subset of the Phobos LIFE organisms, *B. subtilis* MW01, *D. radiodurans* R1, *P. furiosus*, *H. marismortui*, and two species of tardigrades (*Richtersius coronifer* and *Macrobiotus cf. hufelandi*, the latter of which actually was not part of Phobos LIFE), were flown in which actually was not part of Phobos LIFE), were flown in the same Delrin sample tubes used for Phobos LIFE. Rather than placing the organism-loaded Delrin sample tubes inside a discoid biomodule, however, the tubes used in Shuttle LIFE were inserted into larger tubes as part of the CREST-1 commercial payload. Shuttle LIFE was to serve as a dry run for Phobos LIFE operational procedures. Additionally, the science provided from the organisms in LEO would have been used for comparison with results of Phobos LIFE. Since they



FIG. 6. Loading freeze-dried organisms into Phobos LIFE sample tube.

also may be useful for any new LIFE experiments on upcoming deep space missions, we are preparing for publication the Shuttle LIFE postflight results.

6. Study of Life on the Asteroid Redirect Mission and the Future

As part of a NASA Asteroid Redirect Mission (ARM) Broad Agency Announcement, NASA selected a Planetary Society study of accommodating LIFE on ARM. The concept was to fly one or more LIFE biomodules near the exterior of the ARM robotic spacecraft. Then, a few years later, when astronauts traveled to the retrieved piece of an asteroid, they would also remove the LIFE biomodule (or biomodules) for return to Earth. This would have achieved the core science goal of Phobos LIFE of flying organisms in a simulated meteoroid in deep space for several years then returning them to Earth for analysis.

The ARM LIFE study (Betts and Friedman, 2015) found that LIFE could be accommodated on ARM and returned by astronauts. The accommodation was found to be relatively easy and could meet the safety requirements of being retrieved by astronauts. LIFE, in part due to its rugged, flight-tested design, would place very few requirements on the ARM or the crewed mission. Requirements would include placing the biomodule near or on the surface of the spacecraft to avoid high radiation shielding or amplification effects, and keeping the biomodules at a temperature less than 40°C, though very brief higher external temperature spikes could occur as long as the biological samples would not incur significant temperature spikes. For the astronaut retrieval, easy access would also be required, as well as pos-

sibly a tether. Two possible nominal test accommodations were found, one inside and one outside the ARM toolbox that would carry tools for future astronauts.

ARM LIFE was formally proposed to fly on the ARM mission, but the ARM mission was cancelled before selections were made. Though the ARM mission was cancelled, the ARM LIFE accommodation studies illustrated the flexibility of the LIFE biomodule for flying on a variety of missions. The LIFE team is still very interested in flying on future deep space sample returns. We continue to pursue possibilities.

7. Conclusions

Phobos LIFE on the Phobos Sample Return (Grunt) mission would have provided the first study of organism survival after deep space flight beyond Earth's magnetosphere for more than a few days. Although the Phobos Grunt mission failed before reaching deep space, Phobos LIFE can serve as a model for future deep space sample return missions. We are studying flying LIFE on possible future sample return missions. Using the same LIFE biomodule design, or a similar design, such missions may provide new insights into the plausibility of the short transfer of life over relatively small interplanetary distances by testing survivability during transport through deep space inside a simulated meteoroid.

More information on the LIFE experiments can be found at <http://planetary.org/programs/projects/life/>

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

ARM = Asteroid Redirect Mission
 ATCC = American Type Culture Collection
 LEO = low Earth orbit
 LIFE = Living Interplanetary Flight Experiment
 TLDs = thermoluminescence detectors