Maximizing Science Return from Future Rodent Experiments on the International Space Station (ISS): Tissue Preservation. S.Y. Choi, S. Lai, R. Klotz, Y. Popova, K. Chakravarty, J. E. Beegle, C.L. Wigley, and R.K. Globus. NASA Ames Research Center, Moffett Field, CA 94035, USA.

To better understand how mammals adapt to long duration habitation in space, a system for performing rodent experiments on the ISS is under development; Rodent Research-1 is the first flight and will include validation of both on-orbit animal support and tissue preservation. To evaluate plans for on-orbit sample dissection and preservation, we simulated conditions for euthanasia, tissue dissection, and prolonged sample storage on the ISS, and we also developed methods for post-flight dissection and recovery of high quality RNA from multiple tissues following prolonged storage *in situ* for future science return. Livers and spleens from mice were harvested under conditions that simulated nominal, on-orbit euthanasia and dissection procedures including storage at -80˚C for 4 months. The RNA recovered was of high quality (RNA Integrity Number, RIN>8) and quantity, and the liver enzyme contents and activities (catalase, glutathione reductase, GAPDH) were similar to positive controls, which were collected under standard laboratory conditions. We also assessed the impact of possible delayed on-orbit dissection scenarios (off-nominal) by dissecting and preserving the spleen (RNAlater) and liver (fast-freezing) at various time points post-euthanasia (from 5 min up to 105 min). The RNA recovered was of high quality (spleen, RIN>8; liver, RIN>6) and liver enzyme activities were similar to positive controls at all time points, although an apparent decline in select enzyme activities was evident at 105 min. Additionally, various tissues were harvested from either intact or partially dissected, frozen carcasses after storage for ~2 months; most of the tissues (brain, heart, kidney, eye, adrenal glands and muscle) were of acceptable RNA quality for science return, whereas some tissues (small intestine, bone marrow and bones) were not. These data demonstrate: 1) The protocols developed for future flight experiments will support science return despite delayed preservation post-euthanasia or prolonged storage, and 2) High-quality RNA samples from many different tissues can be recovered by dissection following prolonged storage of the tissue *in situ* at -80˚C. These findings have relevance both to high-value, ground-based experiments when sample collection capability is severely constrained, and to future spaceflight experiments that entail on-orbit sample recovery by the ISS crew.

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