Biospecimen Retrieval from NASA’s Rodent Research-1: Maximizing Science Return from Flight Missions


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Background

- Spaceflight experimentation poses various challenges:
  - Limited number of samples
  - Limited number of flights
  - Limited capability to process tissues on orbit

- Biospecimen Sharing Program (BSP) was developed over 50 years ago by the NASA Ames Research Center to maximize science return
Background (Cont’d)

• Ground-based tests were performed to maximize science return from the Rodent Research-1 Validation flight
Rodent Research-1

• Launched on SpX4: September 21, 2016

• 20 female adult mice:
  o 10 for NASA’s validation mission
  o 10 for the National Lab’s science experiment

• Mission duration on the ISS:
  o 33 days (NASA’s Validation)
  o 16-17 days (Novartis)

• Animals euthanized and stored at <-80°C on the ISS until return to Earth on SpX5 (Feb 2015)

• BSP dissection
  o First thaw: April 2015
  o Second thaw: March 2016
Rodent Research-1 BSP

• Ground-based tests were performed to maximize science return from the Rodent Research-1 Validation flight:
  – Tissue Preservation Test: assess the quality of tissues collected from frozen carcasses
    • Results presented at 2014 ASGSR
    • Manuscript recently accepted (PLOS One, 2016)
  – Additional tissue quality test to optimize science return from the RR-1 BSP dissections
    ➢ Carcasses were frozen using the on-orbit timelines for RR-1 (NASA’s Validation flight)
RR-1 First Thaw BSP Dissection

- Carcasses were euthanized and stored at -80°C for over 3 months on ISS and for ~2 months at Ames upon return to Earth (total > 5 months at -80°C)

- 32 different types tissues were retrieved from 40 mice including 10 mice each from flight, ground controls, baseline and vivarium controls, yielding total of 3280 vials of tissues
RR-1 First Thaw BSP Dissection (cont’d)

• BSP tissues have been distributed to the scientific community through the Ames Life Science Data Archive (LSDA)
  ➢ To locate available biospecimens, visit the LSDA at:
    http://lsda.jsc.nasa.gov

• Select samples were provided to Russian research colleagues at the Institute for Biomedical Problems (IBMP)

• NASA GeneLab project: Liver samples were provided for “omics” analyses (transcriptomics, epigenetics and proteomics)
  ➢ Some of the data are currently publically available:
    https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=RR1
**RR1 BSP Dissection workflow**

**Dissector 1**

- Thaw frozen carcass

**Dissector 2**

- Dorsal Skin
- Eyes
- Brain

**Left or Right hindlimb:**
- Gastrocnemius
- Soleus
- Tibialis anterior (TA)
- Extensor digitorum longus (EDL)
- Quadriceps

**Other hindlimb:**
- Gastrocnemius
- Soleus
- Tibialis anterior (TA)
- Extensor digitorum longus (EDL)
- Quadriceps

**Femur**

**Tibia**
BSP Dissection workflow (cont.)

Adrenal glands
Kidneys
Gonadal fat
Ovaries
Uterus
Diaphragm
Thymus

Heart and Lung

Wrap carcass (END)
(total dissection time: ~37-42 minutes)

Liver
Spleen

Process Liver lobes

Process GI tract:
Stomach
Duodenum
Jejunum
Ileum
Cecum
Large intestine
Mesenteric fat
Feces

Process heart chambers
Process lung lobes
Tissue Quality Testing: assess RNA quality of various tissues collected from frozen carcasses (1st thaw dissection)
RR1 Second Thaw BSP Dissection

– 40 carcasses previously dissected during the first thaw dissection (10 per group from flight, ground controls, vivarium and baseline) and stored for about 11 months at Ames Biospecimen Sharing Facility freezer (-80°C) were dissected.

– Additional 7 different types of tissues were retrieved from each of the 40 remaining carcasses.
## Second Thaw Tissues

<table>
<thead>
<tr>
<th>Tissues</th>
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<tbody>
<tr>
<td>Pelvis</td>
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<tr>
<td>Spine</td>
</tr>
<tr>
<td>Aorta</td>
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<tr>
<td>Forearms and Tail</td>
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<tr>
<td>Vagina</td>
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<tr>
<td>Brown Adipose Tissue</td>
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<tr>
<td>Neck muscles</td>
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Tissue Quality Test for the RR-1 second thaw dissection

• Objective:
  – To assess if the tissues collected from the second thaw dissection are viable

• Methods:
  – Tissues were collected from frozen carcasses that were subjected to 2 cycles of freezing and thawing
  – Timelines simulated the on-orbit RR-1 procedures and the first thaw dissection to assess the quality of the second thaw tissues.
  – RIN (RNA integration number) values of select tissues, including kidney, brain, diaphragm, white adipose tissue (WAT) and brown adipose tissue (BAT) were determined as a measure of tissue quality
Adipose Tissues

Brown Adipose Tissue (n=10)  White Adipose Tissue (n=5)

RIN

- first thaw
- second thaw
Brain

![Graph showing RIN values for first and second thaw with error bars and mean +/- SD (n=5).]
Kidneys

- First thaw (n=3)
- Second thaw (n=4)
Diaphragm

First Thaw

Second Thaw

Mean +/- SD (n=5)
Feedback from BSP PIs about the quality of tissues recovered from the second thaw

• Vaginal walls:
  – “The histology is fantastic - they look similar to freshly fixed tissue which is remarkable considering the vaginal walls were frozen, thawed, frozen again and then thawed before fixation” - Dr. Lane Christenson, University of Kansas Medical Center

• Aorta:
  – “RNA isolated from the second thaw aorta were not of high quality for RNA-seq but were suitable for microarray analysis using the FFPE (formalin-fixed, paraffin-embedded) method” - Dr. Sonja Schrepfer, UCSF

• Brown adipose tissue:
  – “PCR array targeted for adipogenic genes were successfully performed using RNA samples isolated from brown adipose tissues” - Dr. Russel Turner, Oregon State University
Summary and Conclusion

• Select tissues can be utilized for gene expression and histology studies despite being retrieved from carcasses subjected to at least two freezing and thawing cycles

• These results expand science return from valuable and limited rodent experiments in space
Acknowledgements

• Rodent Research Project Team, ARC
• Space Biology Program
• ISS Program
EXTRA
Rodent Research-3

• Launched on SpX8: April 8, 2016

• 20 female adult for Eli Lilly/US National Laboratory

• Mission duration: 6 weeks on the ISS

• Animals euthanized and stored at <-80°C on the ISS until return to Earth on SpX9 (August 2016)

• BSP dissection: Sept 2016
RR3 BSP dissection

- Over 25 types of tissues were retrieved from 30 non-treated RR3 mice for NASA BSP

- ~1800 vials of tissues were transferred to Ames Life Science Data Archive for the scientific community (not including the tissues processed by the RR3 PIs and SLPS PI team)

- Tissues were snap frozen, preserved in RNAlater or fixed for histology.