Tissue Preservation Assessment
Preliminary Results:

Speakers: Ruth Globus and Sylvain Costes

RR Workshop at ASGSR meeting
Oct. 2017
Outline: Globus

• Pre-flight ground-based testing done (Choi et al)
  • For gene expression (RIN values)
  • For protein
• Protocols/methods of preservation to date: freezing profiles from cold stowage
• Overview results from RR1- validation
  • RIN values
  • qRT-PCR and enzyme levels
• Summary of findings on sample quality from BSP-PI’s
Pre-flight groundbased testing: Choi et al. PlosOne 2016

• Objectives:
  • Designed to test potential delays in on-orbit dissections and preservation
  • “Bonus” science: worked out conditions to preserve carcass (intact or dissected) under fast-freeze conditions
RR1 Experimental Design for on-orbit sample retrieval for Validation

Primary objective RR1 Validation Mission to demonstrate:
Liver-dissection and fast freezing
Spleen-dissection and preservation in RNALater

31-2 days in space

NASA VALIDATION MICE
10 C57BL/6J mice
(female, 16wk old)

2 mice

Dissect for Spleen + Liver

8 mice

Freeze intact for Body weights & sample retrieval post-flight

21-22 days in space

5 CASIS Control (WT) strain
5 CASIS Genetically modified strain (MuRF1 KO)
(female, 32 wk old)

Hind limb

10 legs

Formalin-fixed

Liver and Spleen Dissected for Validation

5 WT mice
On orbit sample freezing methods
Cold Stowage kit to freeze samples (livers and carcasses) in MSG

**Mini Coldbag**
- Passive low temperature science storage. MCB was not pre-chilled.

**Ice Bricks**
- Solid-liquid phase change material in a hard plastic rectangular container compatible with the cold stowage systems. Ice bricks were pre-chilled in the glacier (~150°C)
- 3 ice bricks were used for each mini coldbag.
Carcasses were wrapped with two layers of aluminum foil and placed in a ziploc bag.

Placed in MCB containing 3 ice bricks.

5 Carcasses were placed in metal box and transferred to MELFI.

Notes: ice bricks are swapped out midday.
RR1: Livers and Spleens were dissected on orbit

Ice blocks to freeze the livers (prechilled in Glacier)

Spleens preserved in RNAlater (4C for 24+ hours then stored in Melfi)
RR1 samples were stored in MELFI until return to Earth on SpX5

On orbit: MELFI (-95°C)  Ground: -80°C
## Freezing Rates (RR1 vs. RR2-4)

### RR1: MCB with 3 ice bricks

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mass (g)</th>
<th>Time to Reach (-20^\circ C) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 Sample 1</td>
<td>22.0</td>
<td>31</td>
</tr>
<tr>
<td>Day 1 Sample 2</td>
<td>22.3</td>
<td>37</td>
</tr>
<tr>
<td>Day 1 Sample 3</td>
<td>21.9</td>
<td>52</td>
</tr>
<tr>
<td>Day 1 Sample 4</td>
<td>23.6</td>
<td>71</td>
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<tr>
<td>Day 1 Sample 5</td>
<td>22.5</td>
<td>83</td>
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<td>Day 2 Sample 1</td>
<td>26.1</td>
<td>36.5</td>
</tr>
<tr>
<td>Day 2 Sample 2</td>
<td>21.9</td>
<td>32.5</td>
</tr>
<tr>
<td>Day 2 Sample 3</td>
<td>21.4</td>
<td>43</td>
</tr>
<tr>
<td>Day 2 Sample 4</td>
<td>23.2</td>
<td>57</td>
</tr>
<tr>
<td>Day 2 Sample 5</td>
<td>22.4</td>
<td>64.5</td>
</tr>
</tbody>
</table>

### RR2-RR5: Direct MELFI insertion

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mass (g)</th>
<th>Time to Reach (-20^\circ C) (min)</th>
<th>Time to Reach (-80^\circ C) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>33.6</td>
<td>38</td>
<td>101.5</td>
</tr>
<tr>
<td>Sample 2</td>
<td>28.14</td>
<td>38</td>
<td>106.5</td>
</tr>
<tr>
<td>Sample 3</td>
<td>26.97</td>
<td>41</td>
<td>87.5</td>
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<tr>
<td>Sample 4</td>
<td>25.33</td>
<td>37</td>
<td>175.5</td>
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<tr>
<td>Sample 5</td>
<td>29.9</td>
<td>40</td>
<td>142</td>
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<tr>
<td>Sample 6</td>
<td>30.61</td>
<td>43.5</td>
<td>108</td>
</tr>
<tr>
<td>Sample 7</td>
<td>26.5</td>
<td>39.5</td>
<td>88.5</td>
</tr>
<tr>
<td>Sample 8</td>
<td>24.1</td>
<td>37.5</td>
<td>77</td>
</tr>
</tbody>
</table>

Mouse carcass times

Data provided by JSC Cold Stowage
**RR-1 First Thaw BSP Dissection**

- *Manuscript describing sample quality published:

- 32 different types of 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

- BSP tissues have been distributed to the scientific community through the Ames Life Science Data Archive (LSDA)

- 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)
RR1 Validation results
RNA quality (RIN) RR1: validation samples

**On-orbit Dissected Liver**

- Positive Control (n=3)
- Basal (n=2)
- Vivarium (n=2)
- Ground Control (n=2)
- Flight (n=2)

**Frozen Carcass Liver**

- Basal (n=7)
- Vivarium (n=6)
- Ground Control (n=6)
- Flight (n=6)
Normalized to L19 housekeeping gene.
Liver samples analyzed in triplicate.
Within group variability reflects animal-to-animal differences
RR1 Liver enzyme results from Validation and Experimental Mice
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Analysis</th>
<th>BSP dissection(s)</th>
<th>Tissue quality acceptable?</th>
<th>IS FCR acceptable as a process</th>
<th>Acceptable with improved sample processing?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>Histology</td>
<td>RR-1 rounds 1 and 2</td>
<td>Some, but not all</td>
<td>RR1 round 1: acceptable, RR1 round 2: not acceptable</td>
<td>Potentially</td>
</tr>
<tr>
<td></td>
<td>qPCR</td>
<td>RR-1 rounds 1 and 2</td>
<td>Some, but not all</td>
<td>Acceptable</td>
<td>Yes</td>
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<tr>
<td>Blood vessel</td>
<td>Transcription profiling, protein expression profiling, histology, immunohistochemistry, Western blot, mechanical properties</td>
<td>RR-1 round 1, RR-3</td>
<td>Some, but not all</td>
<td>Acceptable</td>
<td>Yes</td>
</tr>
<tr>
<td>Bone</td>
<td>Histology, micro CT, qPCR</td>
<td>RR-1 round 1</td>
<td>Some, but not all</td>
<td>Acceptable only in cases where crew time for on orbit dissection is unavailable</td>
<td>Yes</td>
</tr>
<tr>
<td>Tissue</td>
<td>Analysis</td>
<td>BSP dissection(s)</td>
<td>Tissue quality acceptable?</td>
<td>IS FCR acceptable as a process</td>
<td>Acceptable with improved sample processing?</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------</td>
<td>-------------------</td>
<td>----------------------------</td>
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<td>--------------------------------------------</td>
</tr>
<tr>
<td>Feces</td>
<td>Microbiome analyses</td>
<td>RR-3</td>
<td>Some, but not all</td>
<td>Not acceptable</td>
<td>No</td>
</tr>
<tr>
<td>Gut</td>
<td>Transcription profiling, protein expression profiling, histology, immunohistochemistry, Western blot, mechanical properties</td>
<td>RR-3</td>
<td>Some, but not all</td>
<td>Not acceptable</td>
<td>Potentially</td>
</tr>
<tr>
<td>Liver</td>
<td>Histology, qPCR</td>
<td>RR-1 round 1</td>
<td>Some, but not all</td>
<td>Acceptable</td>
<td>Yes</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Transcription profiling, protein expression profiling, histology, immunohistochemistry, Western blot, mechanical properties</td>
<td>RR-3</td>
<td>Some, but not all</td>
<td>Acceptable</td>
<td>Yes</td>
</tr>
<tr>
<td>Spleen</td>
<td>RNA-seq, ELISA</td>
<td>RR-1 round 1</td>
<td>Yes</td>
<td>Not acceptable</td>
<td>Potentially</td>
</tr>
</tbody>
</table>
Summary and Conclusion

• Select tissues can be utilized for gene expression, protein and histology studies, despite being retrieved from frozen carcasses
  • Further analysis needed

• These results expand potential science return from valuable and limited rodent experiments in space
Links/Resources

Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era
(National Research Council, 2011)
http://www.nap.edu/catalog/13048/recapturing-a-future-for-space-exploration-life-and-physical-sciences

CASIS/National Lab
http://iss-casis.org/

Space Biosciences Division-NASA Ames Research Center
http://www.nasa.gov/ames/research/space-biosciences

NASA Life Sciences Data Archive
http://lsda.jsc.nasa.gov

NASA’s Genelab project
http://genelab.nasa.gov

Novartis: Mice in Space video, RR1 study
https://www.youtube.com/watch?v=1L868FzjF2I

The Health Risks of Extraterrestrial Environments (space radiation risk website)
http://three.usra.edu/#section=main
EXTRA
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Ground-Based Tissue Quality Test for the RR-1 second thaw dissection

• Objective:
  • To assess if the tissues collected from the second thaw dissection are of high quality for analysis of gene expression

• Methods:
  • Tissues were collected from frozen carcasses that were subjected to 2 cycles of freezing and thawing
  • RIN (RNA integration number) values of select tissues
  • Additional 7 different types of tissues were retrieved from each of the 40 remaining carcasses.
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

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

• 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
RR3 BSP dissection

• Over 25 types of tissues were retrieved from 30 non-treated mice (10 mice each from Basal, Ground controls and Flight) for NASA BSP and GeneLab

• ~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 PIs)

• Tissues were snap frozen, preserved in RNAlater or fixed for histology.
Temperature profile: shipping from JSC to Ames

ARC_Box_1_NASA_11-13-14

P48404 - Temperature (°C)
Temperature profile between MELFI insertion of samples and MELFI to Glacier transfer for return
Temperature profile during return to Earth
## RR1: Tissue freezing rates using Cold block bricks in MCB

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<tr>
<th>Sample</th>
<th>Mass (g)</th>
<th>Time to Reach -20°C (sec)</th>
<th>Time to Reach -80°C (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1-1</td>
<td>2.91</td>
<td>100</td>
<td>225</td>
</tr>
<tr>
<td>Sample 1-2</td>
<td>3.05</td>
<td>110</td>
<td>180</td>
</tr>
<tr>
<td>Sample 1-3</td>
<td>2.97</td>
<td>150</td>
<td>290</td>
</tr>
<tr>
<td>Sample 1-4</td>
<td>3.14</td>
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<td>285</td>
</tr>
<tr>
<td>Sample 1-5</td>
<td>2.88</td>
<td>145</td>
<td>395</td>
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<td>Sample 2-7</td>
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<tr>
<td>Sample 2-8</td>
<td>1.6</td>
<td>170</td>
<td>640</td>
</tr>
</tbody>
</table>

Data provided by JSC Cold Stowage