Temporal RNA Integrity Analysis of Archived Spaceflight Biological Samples from ALSDA from 1991 to 2016

Elizabeth Delgadillo Talburt[1], Alison J. French[2], Danielle K. Lopez[2], San-Huei Lai Polo[2], Valery Boyk[2], Marie T. Dinh[2], Jon C. Rask[2], Kaushik Chakravarty[3], Helen J. Stewart[4]

NASA Volunteer Internship Program[1], Wyle Labs[2], Logyx LLC[3], NASA AMES Research Center[4]

National Aeronautics and Space Administration

Abstract

The purpose of this study is to assess the quality of spaceflight tissues stored in Ames Life Sciences Data Archive (ALSDA) freezers. To assess the viability of a select group of tissues, RNA integrity number (RIN) values were calculated for RNA extracted from rodent livers. It was found that mean RIN values from Commercial Biomedical Test Module 3 (CBTM-3), Rodent Research 1 (RR1), and Rodent Research 3 (RR3) were suitable for downstream functional analysis (RIN > 5) while the mean RIN value for SLS-1 was not (RIN = 2.5 ± 0.1).

Background

ALSDA freezers hold thousands of biospecimens that vary in tissue type, organ, and animal model. Other factors affecting tissue viability include dissection procedures, fixatives, age, and specimen containers.

Table 1: Indicates payload , storage and corresponding species included in this study.

Year	Payload	Species	Experimental Group	Fixative
1991	SLS-1	Rat	Ground	LN_2
2011	CBTM-3	Mouse	Ground, flight, & basal	LN_2
2014	RR1	Mouse	Ground & flight	LN_2
2016	RR3	Mouse	Ground & flight	LN_2 & RNAlater

Table 1 above describes the payload and corresponding species and lists pertinent information that may have an effect on the RNA quality.

Methods

RNA extraction

RNA extractions from SLS-1 and RR3 (RNA Later preserved) liver samples were performed by the ALSDA. GeneLab had previously performed RNA extractions on CBTM-3, RR1, and RR3 livers preserved in LN2. All RNA extractions were performed in accordance with the QIAGEN DNA/RNA AllPrep Mini Handbook.

Figure 1: Experimental design flow

Extract RNA → Calculate RIN → Compare RIN → Statistical Analysis

RIN Value Determination

RIN values were calculated using the extracted RNA. All RIN values were determined using the Agilent Bioanalyzer 2100 system

Statistical Analysis

All statistical analyses were performed using Prism 6.07 software analysis tools.

The following results were compared:

- Mean RIN values for each payload
- Mean RIN values for each experimental group (flight, ground, and basal) within a payload
- Mean RIN values comparing the fixative used for payload RR3

Figure 2 shows the results for mean RIN values per payload. Experimental group had no effect on RIN within a payload (Figure 3). For RR3 specimens, RNA later fixed tissues had a higher RIN value than tissues fixed in LN_2 (Figure 3).
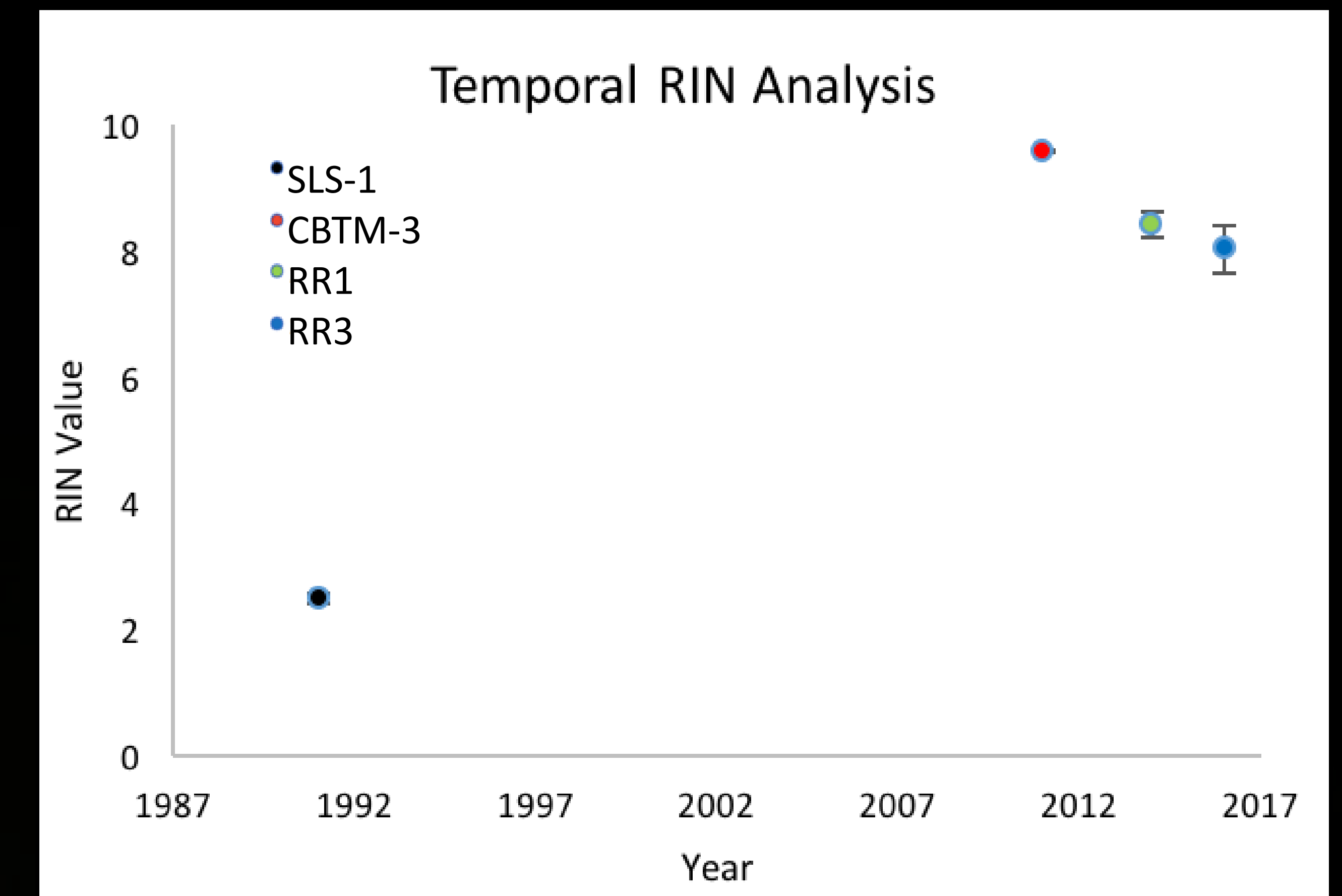
Results

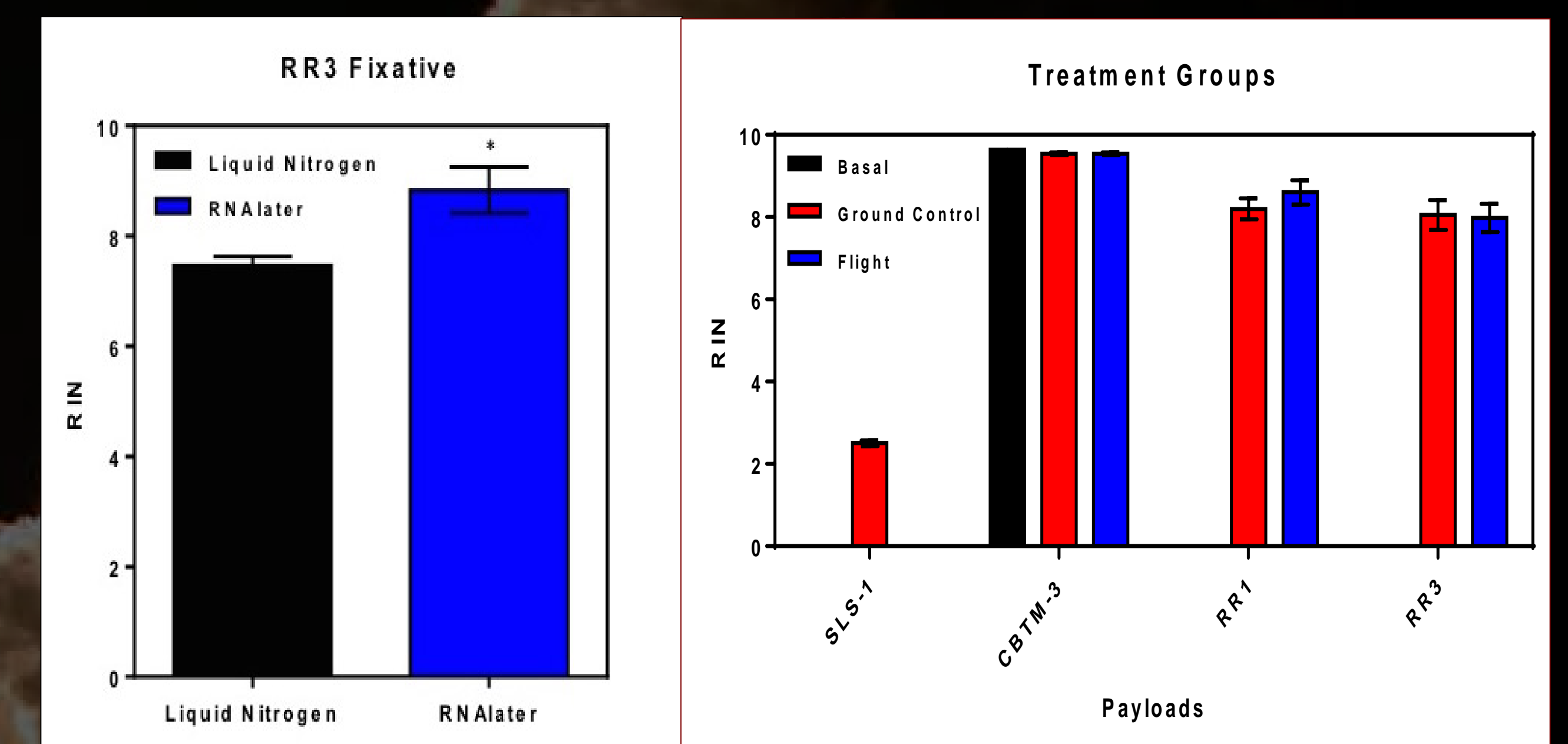Figure 2: Temporal comparison of RIN values from 1991 to 2016. Error bars represent SEM.

Figure 3: Comparison of mean RIN values for LN_2 fixed RR3 tissues and RNA later fixed RR3 tissues (left) and mean RIN values between experimental groups within each payload (right).

Conclusion

RIN values differ between biospecimens held in ALSDA freezers. These differences could be attributed to the age, storage method, and fixative used on these tissues. Additional work should be done to expand this study to other organ types, animal models, and to include histopathological testing.