Vacuum Packed Transport maintains the RNA quality

Martina Farronato, Dea Filippini, Claudia Rossetti, Borislav Rusev, Rita T. Lawlor, Aldo Scarpa

ARC-NET APPLIED RESEARCH ON CANCER, VERONA-ITALY

INTRODUCTION

Prolonged transport time and processing delays of tissue specimens affect RNA quality.

The aim of this study was to test the impact of storing tissue samples under vacuum condition prior to processing on RNA integrity.

We first tested the integrity of RNA extracted from 12 samples of murine tissue from the strain of nude mice Swiss-nu/nu.

Fig.1: Athymic mice Swiss-nu/nu.

The results obtained from murine samples were then validated using samples of normal tissue from patients who underwent surgical resection for adenocarcinoma of the pancreas.

MATERIALS AND METHODS

Samples: A total of 24 tissue samples were taken from 12 mice SWISS-nu/nu mice, one liver sample and one pancreas sample from each. One normal pancreas sample (hNP) was taken from each of two patients who underwent surgical resection.

Fig.2: Tissue Vacuum (Kaltek)®

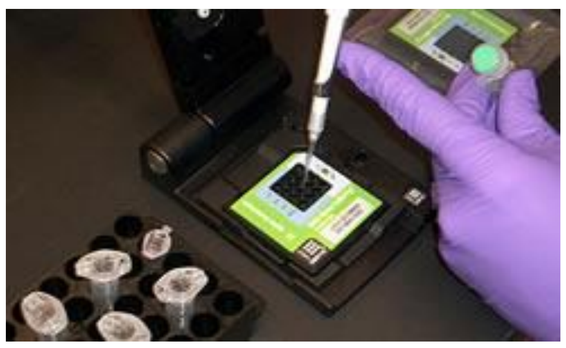


Fig.3: RNA chip.

Instruments: Tissue Vacuum (Kaltek)®, AllPrep DNA/RNA Mini Kit (Qiagen), Agilent2100 Bioanalyzer, Kit Agilent RNA 6000 nano Reagent Part 1.

Test of Vacuum Storage Mice Tissues

Matched liver and pancreas tissues from 12 mice were used to test vacuum storage conditions at different time points. Tissues were treated as follows: (i) 4 were immediately snap-frozen after collection, (ii) 4 were put under vacuum in a refrigerator at 4°C for 16 hours prior to freezing and (iii) 4 were stored for 40 hours prior to freezing.

Test of Vacuum Storage HumanTissues

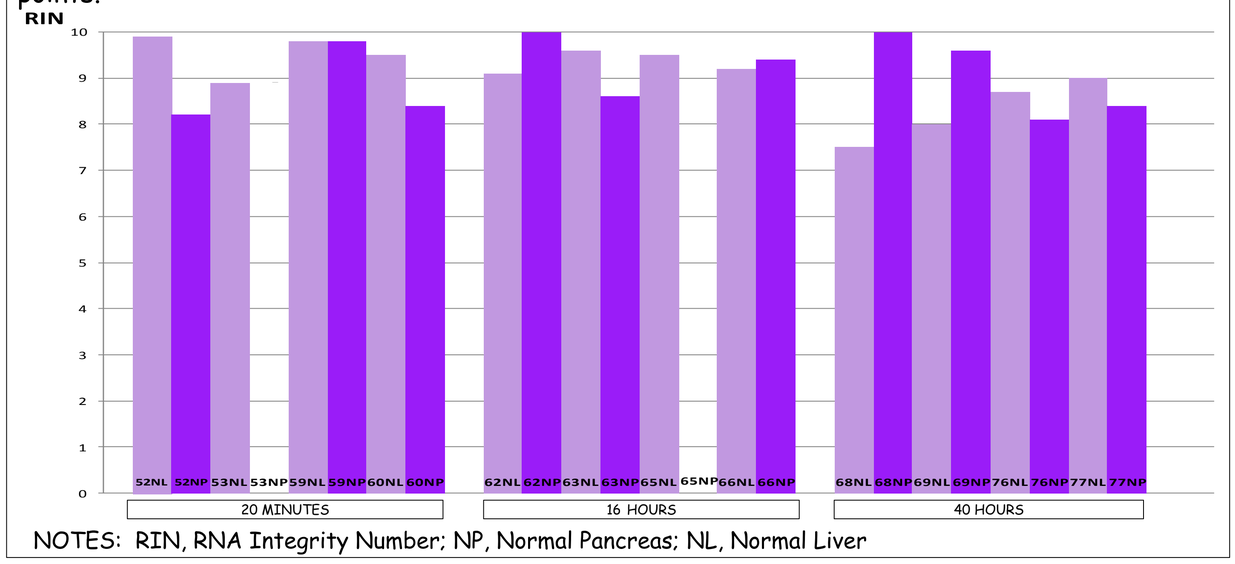
Two hNP were each cut into three smaller specimens upon collection and storage conditions determined by the results from the mice specimens: (iv) 2hNP were immediately snap-frozen, (v) 2hNP were put under vacuum in a refrigerator for 48 hours prior to freezing, (vi) 2hNP were put under vacuum in a refrigerator for 115 hours prior to freezing procedure.

RNA was isolated using the All-Prep DNA/RNA Minikit and was assessed with Agilent 2100 Bioanalyzer.

RESULTS

All murine samples showed RIN values greater than 8 regardless of time delay from collection to storage, indicating that RNA integrity is guaranteed in up to 40 hours vacuum storage a 4°C.

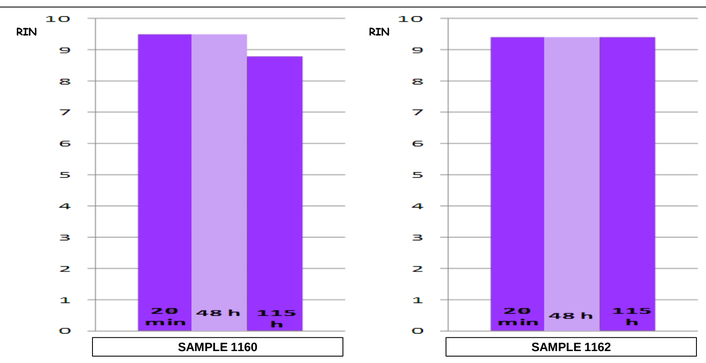
Fig. 4: Quality of RNA in murine samples preserved under vacuum at + 4°C at different time points.



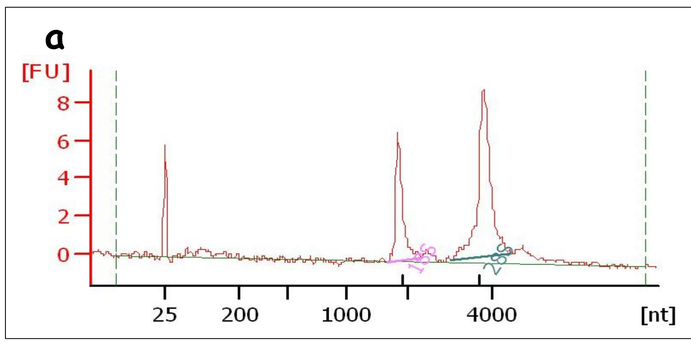
NOTES: RIN, RNA Integrity Number; NP, Normal Pancreas; NL, Normal Liver

Based on the RIN integrity of vacuum-stored mouse tissues, two hNP tissue specimens were stored for up to 115 hours under vacuum and RNA integrity was subsequently checked.

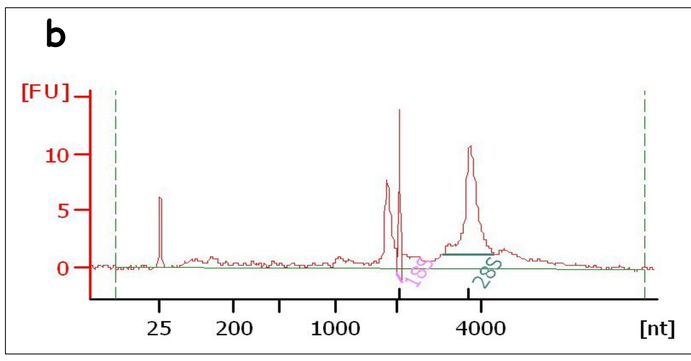
Fig. 5: RNA integrity in human pancreatic samples preserved under vacuum at +4°C at different time points.



The results confirmed that the RNA integrity is assured.



SPECIMEN 62 NP
RIN: 10
RNA Area: 49.3
RNA Concentration: 43 ng/µl
rRNA Ratio [28s / 18s]: 2.0



SPECIMEN 65 NP
RIN: N/A
RNA Area: 97.4
RNA Concentration: 84 ng/µl
rRNA Ratio [28s / 18s]: 5.1

Fig.6: Electropherogram detailing the regions that are indicative for RNA quality; **a** example of intact RNA; **b** example of degraded RNA

CONCLUSIONS

This study indicates that: (1) the temporary storage of tissue under-vacuum at 4 °C ensures RNA integrity for up to 115 hours; (2) Fresh tissues may be conserved for longer periods thus permitting transport of fresh surgical specimens between distant institutes;

Beyond these findings, the study also suggests that, once cell viability has been evaluated, vacuum stored samples may be used to establish primary cell cultures and xenografted mice thus delaying the need for immediate intervention after sample retrieval.

An important indirect implication of the storage method is the possibility of replacing conventional formalin fixation as main storage procedure for transport of diagnostic materials from Surgery to Pathology thus eliminating the need for this toxic substance in operating theatres.